THE UNIVERSITY
of EDINBURGH

Regional Brain Volumes and Antidepressant Treatment Resistance in Major Depressive Disorder

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Submitted to the University of Edinburgh for the degree of

Doctor of Philosophy

September 2017

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This work is dedicated to the memory of my Aunt, Marilyn Anne Machin (1947-2016). From the Hotel d'Eiffel to doofah-on-the-head there was never a dull moment.

Abstract

Major depressive disorder (MDD) is a heritable and highly debilitating condition with antidepressants, first-line treatment, demonstrating low to modest response rates. No current biological mechanism substantially explains MDD but both neurostructural and neurochemical pathways have been suggested. Further explication of these may aid in identifying subgroups of MDD that are better defined by their aetiology. Specifically, genetic stratification provides an array of tools to do this, including the intermediate phenotype approach which was applied in this thesis. This thesis explores genetic overlap with regional brain volume and MDD and the genetic and non-genetic components of antidepressant response.

The first study utilised the most recent published data from ENIGMA (Enhancing Neuroimaging Genetics through Meta-analysis) Consortium's genome-wide association study (GWAS) of regional brain volume to examine shared genetic architecture between seven subcortical brain volumes and intracranial volume (ICV) and MDD. This was explored using linkage disequilibrium score regression (LDSC), polygenic risk scoring (PRS) techniques, Mendelian randomisation (MR) analysis and BUHMBOX (Breaking Up Heterogeneous Mixture Based On Cross-locus correlations). Results indicated that hippocampal volume was positively genetically correlated with MDD (r_g= 0.46, P= 0.02), although this did not survive multiple comparison testing. Additionally, there was evidence for genetic subgrouping in Generation Scotland: Scottish Family Health Study (GS:SFHS) MDD cases (P=0.00281), however, this was not replicated in two other independent samples. This study does not support a shared architecture for regional brain volumes and MDD, however, provided some evidence that hippocampal volume and MDD may share genetic architecture in a subgroup of individuals, albeit the genetic correlation did not survive multiple testing correction and genetic subgroup heterogeneity was not replicated.

To explore antidepressant treatment resistance, the second study utilised prescription data in (GS:SFHS) to define a measure of (a) treatment resistance (TR) and (b) stages of resistance (SR) by inferring antidepressant switching as non-response. GWAS were conducted separately for TR in GS:SFHS and the GENDEP (Genome-based

Therapeutic Drugs for Depression) study and then meta-analysed (meta-analysis n=4,213, cases=358). For SR, a GWAS on GS:SFHS only was performed (n=3,452). Additionally, gene-set enrichment, polygenic risk scoring (PRS) and genetic correlation analysis were conducted. No significant locus, gene or gene-set was associated with TR or SR, however power analysis indicated that this analysis was underpowered. Pedigree-based correlations identified genetic overlap with psychological distress, schizotypy and mood disorder traits.

Finally, the role of neuroticism, psychological resilience and coping styles in antidepressant resistance was investigated. Univariate, moderation and mediation models were applied using logistic regression and structural equation modelling techniques. In univariate models, neuroticism and emotion-orientated coping demonstrated significant negative association with antidepressant resistance, whereas resilience, task-orientated and avoidance-orientated coping demonstrated significant positive association. No moderation of the association between neuroticism and TR was detected and no mediating effect of coping styles was found. However, resilience was found to partially mediate the association between neuroticism and TR.

Whilst the first study does not indicate a genetic overlap between regional brain volumes and MDD, it demonstrates the utility of the intermediate approach in complex disease. Antidepressant resistance was associated with neuroticism both genetically and phenotypically, indicating its role as an intermediate phenotype. Nonetheless, larger sample sizes are needed to adequately address the components of antidepressant resistance. Further work in antidepressant non-response may help to identify biological mechanisms responsible in MDD pathology and help stratify individuals into more tractable groups.

Lay summary

Major depressive disorder (MDD) is a common and disabling condition. Currently antidepressant medication is the first therapeutic option for individuals with MDD but response rates are low to modest. No current explanations for MDD have adequately explained it but both brain structural changes and neurochemical alterations have been found. Therefore, examining both brain volumes and the antidepressant response (as antidepressants induce chemical changes themselves) could be used to try and explain more about MDD individuals. It is not fully understood whether the genetics of MDD individuals predispose them to smaller brain volumes or this is a result of the environment and/or course of the illness. In this thesis, three studies were completed. The first of these examined whether there was any evidence of overlap in the genes associated with MDD and the genes associated with larger brain volume in seven regions as well as total brain volume. This study found that there was a possible subgroup of MDD patients that had genetic overlap with hippocampus but this finding was not replicated. No other regions demonstrated strong genetic overlap, although to detect weaker correlations, larger sample sizes would be needed. The second study looked at antidepressant resistance using prescription records in a cohort taken from the general Scottish population. The study examined genetic variants and genetic overlap in treatment resistance. Despite this being one of the largest studies to examine treatment resistance, sample sizes were too small to identify genes associated however genetic overlap was demonstrated with neuroticism, psychological distress, schizoptypy and mood disorder traits. Finally, the last study explored the non-genetic factors in antidepressant resistance. This used models to look at the effect of neuroticism, psychological resilience and coping styles in treatment resistance. The study found that neuroticism, resilience and coping styles are all associated with antidepressant resistance and that neuroticism may induce treatment resistance by, in part, decreasing psychological resilience. These studies together demonstrate little evidence for genetic overlap of brain volumes and MDD but show that neuroticism may have an important role in resistance to antidepressants.

Declaration

I declare that this thesis has been composed by me, that the work described in this thesis is my own (except where otherwise stated), and that the work described in this thesis has not been submitted for any other degree or personal qualification.

The thesis includes one article that has been published (the inclusion of this article has been permitted by the journal):

Chapter 3:

Wigmore EM, Clarke TK, Howard DM, Adams MJ, Hall LS, Zeng Y et al., (2016): **Do Regional Brain Volumes and Major Depressive Disorder Share Genetic Architecture? A study in Generation Scotland (n=19,762), UK Biobank (n=24,048) and the English Longitudinal Study of Ageing (n=5,766).** *Transl Psychiatry*. **7**, e1205; doi:10.1038/tp.2017.148.

And three articles submitted for publication:

Chapter 2:

Wigmore EM, Clarke TK, Porteous DJ, Nicodemus KK, McIntosh AM (2017): **Toward genetic stratification of neuropsychiatric disorders.** In submission.

Chapter 4:

Wigmore EM, Hafferty JD, Hall LS, Howard DM, Clarke TK, Adams MJ et al., (2017): **Genome-wide association study of antidepressant treatment resistance in a population-based cohort using health service antidepressant prescription data with meta-analysis with GENDEP.** In submission.

Chapter 5:

Wigmore EM, Navrady LB, Hafferty JD, Clarke TK, Campbell A, Thomson PA et al., (2017): **Antidepressant treatment resistance and the impact of neuroticism, psychological resilience and coping style.** In submission.

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Acknowledgements

First and foremost, I would like to thank my supervisors Professor Andrew McIntosh and Professor Kristin Nicodemus for offering me this PhD and for your continued guidance and support throughout. Andrew, your enthusiasm and encouragement has guided me through all aspects of this PhD and your flexibility around the project has meant that I have been allowed to pursue any avenue of interest. Kristin, your expertise and encouragement has been of great value to me and I have appreciated your support. Additionally, I would also like to thank Dr Toni-Kim Clarke for your advice and guidance, especially at the beginning of my PhD. I would like to thank my supervisor in Aarhus, Professor Manuel Matthiessen for helping me to settle quickly into the new environment and supervising me in my analysis whilst I was there, and Professor Ole Mors for instigating and organising the 4-month stay. I am also grateful to all of my coauthors for all of their contributions to the papers which constitute the chapters in this thesis as well as all those who have offered me academic help including Professor David Porteous, Professor Pippa Thomson and Professor Ian Deary, and those at the Kennedy Tower such as Heather Whalley and Stephen Lawrie.

I would also like to thank all of my friends and colleagues at the Kennedy Tower. It has been such fun to work with all of you and you have guided me through the good, the bad and the ugly with lab whisky, Whine club and trips to the Merlin when they were needed. To Lauren, Jude, Emma, Yanni, Jonathan, Shen, Clara, Lynsey, Toni, David, Mark and Masoud a huge thanks for everything, it would not have been the same without you. Additionally, I would like to thank my colleagues and friends at the IGMM and at Aarhus Universitet for your company and academic support.

Thank you also to all those that offered me support and distraction through these three years, these include Cathy, Katie, Eva, Manon, Paula and numerous others.

Lastly, I would like to thank my family. You have truly been there for me throughout and from the initial interview, first publication and (most recently) a post-PhD job offer you have been there to celebrate every victory, big or small. Thank you.

Abbreviations

ADHD – attention-deficit hyperactivity disorder

AoC – Avoidance-orientated coping

ASD – autism spectrum disorder

AUC – area under the curve

β - beta (regression coefficient)

BNF - British National Formulary

BRS – Brief Resilience Scale

BUHMBOX - Breaking Up Heterogeneous Mixture Based On Cross-locus correlations

CFI - comparative fit index

CI - 95% confidence interval

CISS - Coping Inventory for Stressful Situations

DSM-IV/V - The Diagnostic and Statistical Manual of Mental Disorders (4th/5th edition)

ELSA – English Longitudinal Study of Ageing

ENIGMA – Enhancing NeuroImaging Genetics through Meta-Analysis

EoC – emotion-orientated coping

EPQ - Eysenck personality questionnaire: extraversion

FDR - false discovery rate

GCTA - Genome-wide Complex Trait Analysis

GENDEP - Genome-based Therapeutic Drugs for Depression

GHQ - general health questionnaire

GREML - genomic restricted maximum likelihood

GRM - genetic relationship matrix

GS:SFHS or GS - Generation Scotland: The Scottish Family Health Study

GWAS - genome-wide association study

h^2 - narrow sense heritability estimate from twin/pedigree studies

HRDS – Hamilton Rating Depression Scale

HWE - Hardy-Weinberg equilibrium

ICD-9 - International Classification of Diseases (9th edition)

IVW – Inverse-variance weighted method

LD - linkage disequilibrium

LDSC - linkage disequilibrium score regression

LRT - likelihood ratio test

MAF - minor allele frequency

MARS - Munich Antidepressant Response Study

MADRS - Montgomery–Åsberg Depression Rating Scale

MAOI – monamine oxidase inhibitor

MDD - major depressive disorder

MDQ - mood disorder questionnaire

MDS - multidimensional scaling

MR – Mendelian Randomisation

NA - not available (missing data)

NEWMEDS - Novel Methods Leading to New Medications in Depression and Schizophrenia

NHS – National Health Service

OR - odds ratio

P - associated p-value of test statistic

PC - principal component

PCA - principal components analysis

PGC - Psychiatric Genomics Consortium

PRS - polygenic risk scores

QC/QCd - quality control/quality controlled

R^2 - trait variance explained

r^2 - linkage disequilibrium squared correlation coefficient

RCT – randomised control trial

r_g - genetic correlation

RMSEA - root mean square error of approximation

SCID - structured clinical interview for DSM-IV disorders

SE or s.e. - standard error

SIMD - Scottish Index of Multiple Deprivation

SNP - single nucleotide polymorphism

SNP h^2- SNP heritability estimate

SPQ - schizotypal personality questionnaire

SR – stages of antidepressant resistance

SSRI – Selective Serotonin Reuptake Inhibitor

STAR*D - Sequenced Treatment Alternatives to Relieve Depression

TCA – Tricylic antidepressant

ToC – Task-orientated coping

TLI - Tucker-Lewis index

TR – antidepressant treatment resistance

UKB – UK Biobank

WLSMV - weighted least squares, mean and variance adjusted

Contents

Chapter 1. Introduction.

1.1 Background of MDD

Major depressive disorder (MDD) is a highly debilitating condition that contributes a large proportion of global disability (Ustün, Ayuso-Mateos, Chatterji, Mathers, & Murray, 2004). It is one of the oldest disorders to be formally identified in psychiatry (Jackson, 1986) and remains one of the most prevalent psychiatric disorders, however its aetiology continues to be elusive. Both psychological and drug interventions are used in MDD but antidepressant use is the most common form of treatment. High levels of heterogeneity in MDD and the complex contribution and interactions of genetic and environmental factors are likely meaningful underlying reasons. The economic burden for MDD was estimated at 2.5 trillion US dollars in 2010 with numbers expected to rise substantially in the coming years (Bloom et al., 2012). On an individual-level, those with serious mental illnesses have been found to earn around $16,000 less annually than healthy controls (Kessler et al., 2008). Thus, it is imperative to elucidate more about the mechanisms behind the disorder and develop more efficacious drugs that better target MDD aetiology.

Definition of MDD

Diagnosis of MDD follows the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) (American Psychiatric Association, 2013). It is characterised by five or more depressive symptoms that must all persist within at least a two-week period. Nine possible depressive symptoms are given in DSM-5 criteria; hence multiple combinations of symptoms are inclusive in the same MDD diagnosis likely contributing to the substantial heterogeneity. Moreover, it has been hypothesised that MDD could incorporate multiple subgroups with varying aetiologies or, alternatively, could be a continuum of varying severity (Kendler & Gardner, 1998).

Many subtypes of MDD have been proposed over a number of years. Traditionally, MDD was divided into reactive and endogenous subtypes which refer to stress-induced and stress-absent MDD, respectively. The DSM-IV (which preceded DSM-5) recognised five symptomatic subtypes of MDD: melancholic, atypical, catatonic,

postpartum and seasonal affective disorder. A division between single and recurrent episode depression has also been noted with recurrent depression having a higher incidence of anxiety comorbidity, higher depression scores and decreased perception of satisfactory social support compared with single episode cases (Wilhelm, Parker, Dewhurst-Savellis, & Asghari, 1999). Nevertheless, a systemic review of 20 studies in MDD subtypes concluded that no current evidence supported the use of symptomatic subtypes (van Loo, de Jonge, Romeijn, Kessler, & Schoevers, 2012), consequently research is now directed towards stratifying MDD into more biologically-evidenced groups.

Epidemiology of MDD

In 2011, a cross-national study estimated the 12-month prevalence of major depressive episodes at 5.5% and 5.9% and a lifetime prevalence of 14.6% and 11.1% for high to low-income countries, respectively (Bromet et al., 2011), however lifetime prevalence is subject to recall bias and underestimation (Moffitt et al., 2010; Patten, 2009). A large Canadian study reported that 6.6% of past-year MDD individuals reported a suicide attempt, 4.8% reported alcohol abuse and 33.1% were taking an antidepressant (Patten et al., 2015). Women are twice as likely to be affected than men (Seedat et al., 2009) and have been shown to report a lower age of onset and higher comorbidities of panic disorder with agoraphobia and anxiety whereas men reported higher comorbidities of alcohol dependence/abuse (Schuch, Roest, Nolen, Penninx, & de Jonge, 2014). The similarities between the 12-month prevalence as well as similarities in age of onset and MDD severity in high and low-income countries demonstrates that MDD is an important worldwide phenomenon (Kessler & Bromet, 2013).

Comorbidities

MDD has a large number of comorbidities that include other psychiatric disorders and some non-psychiatric diseases. For the most part, the mechanisms underlying the overlap is unknown although genetic factors are thought to play a role. As classification of many of the psychiatric disorders is based on symptoms only, there is debate as to the legitimacy of these diagnostic boundaries (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013) which could contribute to the observed

overlap. MDD is also known to occur in two common disease groups; cancer, with those affected associated with a decreased likelihood of returning to work (Pirl, Greer, Temel, Yeap, & Gilman, 2009), and cardiovascular disease, with one in five suffering with MDD (Elderon & Whooley, 2013). Furthermore, diseases with co-morbid depression are associated with poorer outcomes. For example, in cardiovascular disease, depression predicts higher mortality and morbidity in congestive heart failure, myocardial infarction and post-surgery patients (Nemeroff & Goldschmidt-Clermont, 2012). Therefore, understanding the basis for these comorbidities could aid in improving patient prognosis.

1.2. Genetics of MDD

Familial aggregation of MDD has been described for decades but it is only until recently that any genome-wide associations have been documented (Mullins & Lewis, 2017). The genetic component of depression has been notoriously complex and difficult to study. Family-based, twin studies and adoption studies are common methods for establishing the existence of a genetic component in disease. However in MDD, no high quality adoption studies have been produced (Flint & Kendler, 2014).

Heritability

Twin studies have estimated narrow-sense heritability of MDD at 37% (P. Sullivan, Neale, & Kendler, 2000). Narrow-sense heritability is the proportion of the trait's phenotypic variance that is attributable to additive genetics. It can be calculated using large family-based samples or twin samples however these are susceptible to contributions of shared environment. To circumvent this, unrelated samples can be used to calculate the single nucleotide polymorphism (SNP) heritability which is the variance explained by common genetic variants. Specifically, SNPs are single nucleotide base changes that occur at specific regions in the genome and are common within the general population. In MDD, the SNP heritability has been calculated at 21% (RipkeWray, et al., 2013). For the majority of diseases (MDD included), the SNP heritability estimates are lower than the narrow-sense heritability estimates and the difference between these has been termed the 'missing heritability'. Explanations for this include over-estimations of narrow-sense heritability caused by shared environment and the unaccounted variance of rare genetic variants, gene interactions

or poorly tagged SNPs in SNP heritability (Wray & Maier, 2014). Understanding the contribution of shared environment could enable more accurate heritability estimates. A recent study by Zeng *et al.*, (2016) measured the contribution of nuclear family, sibling and couple associated environment on MDD in the Generation Scotland cohort study (the main cohort used for this thesis). They found that couple-associated environment accounted for 14% of the phenotypic variance in addition to common variant-associated and pedigree-associated genetics, which accounted for 12% and 35% respectively (Zeng et al., 2016). This therefore illustrates a high contribution of shared couple environment in MDD that may have important implications in the planning of future studies.

Genome-wide association studies

The past few decades of genome-wide association studies (GWAS) in MDD have been less fruitful than in other disease areas until recently, most likely owing to the excessive heterogeneity of the disorder. Until two years ago, the largest case-control GWAS of MDD, completed by the Psychiatric Genomics Consortium (PGC), on over 9,000 cases and over 9,000 controls, presented no genome-wide significantly associated variants. In the same year, the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium completed a GWAS on depressive symptoms on over 34,000 individuals and similarly produced no significant results. Both these studies attributed their lack of findings to insufficient power.

In 2015, the CONVERGE (China, Oxford and Virginia Commonwealth University Experimental Research on Genetic Epidemiology) Consortium published the first GWAS to identify genome-wide significant variants. The GWAS was completed on a homogenous sample of Chinese women (with over 5000 cases and 5000 controls) with severe MDD and identified 2 loci (one near the *SIRT1* gene and the other in the intron of *LHPP*) which both replicated in an independent sample (CONVERGE consortium, 2015). A year later, 23andMe identified 15 loci using self-report data in over 75,000 individuals and found evidence for the enrichment of the *MEIS2* subnetwork which has a known role in hippocampal neurogenesis (Hyde et al., 2016). This significant progress in MDD has highlighted the importance of sample size in heterogeneous disorders. Identifying the genetic underpinnings for MDD may be critical to

understanding its underlying biological mechanism and, with the future availability of even larger samples, it may be possible to ascertain aetiological pathways, biomarkers and therapeutic targets.

Genetic overlap

Further exploration into MDD comorbidities has revealed substantial genetic overlap with other disorders. The PGC conducted a cross-disorder GWAS looking into the shared genetics between five psychiatric disorders: MDD, schizophrenia, autism spectrum disorder (ASD), bipolar disorder and attention deficit-hyperactivity disorder (ADHD) (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). The analysis found four loci in common across the disorders and further identified a role of calcium channel signalling pathways. Additionally, borderline personality disorder has also demonstrated genetic overlap with MDD (Witt et al., 2017). Polygenic risk scores (PRS) produce an individual genetic score for a loading of a trait and can be used to identify genetic overlap. A 2016 Dutch study, assessed the association of PRS of psychiatric and metabolic disorders in MDD and subtypes (typical and atypical) finding that psychiatric and not metabolic disorders shared overlapping architectures and that the two subtypes were partially distinct (Milaneschi et al., 2016). Moreover, mania and MDD demonstrate a genetic correlation of 0.65 (McGuffin et al., 2003), MDD and alcohol disorders have been demonstrated to have a genetic correlation of 0.58 (Olvera et al., 2011) and anorexia nervosa and MDD have a shared genetic variance of 34% (Wade, Bulik, Neale, & Kendler, 2000).

In non-psychiatric traits, a review of prior GWAS meta-analyses in cardiometabolic traits associated the top findings with reported genetic associations in mood disorders (including MDD and bipolar disorder). They identified 24 shared genes between the two disease groups including those involved in the hypothalamus-pituitary-adrenal (HPA) axis, circadian rhythm, inflammation, neurotransmission and metabolism all of which have been previously implicated to be of biological significance in both diseases (Amare, Schubert, Klingler-Hoffmann, Cohen-Woods, & Baune, 2017). Analysis of the lipidome (referring to all lipids in the human body) in MDD, implicated shared genetic aetiology with ether-phosphatidylcholines and omega-6 fatty acid (a precursor to inflammatory mediators) (Knowles et al., 2017). Furthermore, genetic overlap

between type 2 diabetes and MDD was examined in two large GWAS meta-analysis cohorts by identifying overlapping SNPs above the *P*-value cut-off of $<1.0x10^{-7}$. After further applying pathway analysis, the study described immune responses, cell signalling, lipid metabolism and cancer associated pathways shared between the two diseases (Ji, Zhuang, & Shen, 2016). Moreover, genetic correlation has been found between MDD and heart rate variability; rg= -0.23 *P*= 0.02 for ultra-low frequency heart rates (Su et al., 2010). This therefore demonstrates the utility of examining shared genetic architecture in order to understand the pathways underpinning comorbidities.

1.3. Possible Biological Mechanisms

Multiple theories have been given as to the aetiology of MDD, however none so far have adequately explained it. The role of monoamines, the HPA axis, neuroinflammation, other neurotransmitters and neurostructural mechanisms are explored here, although it should be mentioned that these are not the only theories for causality.

Monoamine hypothesis

The monoamine hypothesis is the oldest biological mechanism theorised for depression but poorly replicated results and lack of clinical success has led to its loss of popularity. Monoamines are neurotransmitters in the central nervous system (CNS) that include serotonin, noradrenaline and dopamine. They have a wide array of functions but they have all been implicated in the pathology of MDD (Elhwuegi, 2004). The monoamine theory dates back to the 1950s where the serotonin actions in a hallucinogen, lysergic acid diethylamide (LSD), and an antihypertensive agent, reserpine, were linked to mental health and MDD (Hirschfeld, 2000). Since then research into monoamines and their role in MDD has expanded and prompted the generation of monoamine oxidase inhibitors (MAOIs), and later other antidepressants (further details on antidepressants are given below). Whilst a decrease in monoamines is concurrently found in MDD individuals, animal serotonin knock-out studies have not fully captured depression and current antidepressant classes have had limited success (Belmaker & Agam, 2008). Explanations for this discrepancy include that depression may cause the decrease in monoamines or that another factor is responsible for both the reduction in monoamines and onset of MDD (aan het Rot, Mathew, &

Charney, 2009). It is possible that stress is such a factor as it has been shown to induce a reduction in monoamine levels and is associated with depression onset (Cowen, 2002).

The hypothalamus-pituitary-adrenal (HPA) axis

There is considerable evidence for the involvement of stress in MDD (Tennant, 2002). Stress involves the HPA axis which is regulated by a negative feedback loop. In brief, a stressful event will stimulate the hypothalamus to release corticotropin-releasing hormone (CRH) which binds to receptors on the pituitary gland which in turn releases adrenocorticotropic hormone (ACTH). ACTH then binds to the adrenal gland releasing cortisol which acts on glucocorticoid receptors in the hippocampus that then sends signals to the hypothalamus to decrease CRH. Both cortisol and CRH have been strongly associated with MDD and evidence of elevated levels in stress and trauma have also been shown (Belmaker & Agam, 2008). Nevertheless, the role of stress and the HPA axis has not fully explained the aetiology MDD. Stress levels are not necessarily predictive of a depression outcome and cortisol levels have not been found to be a reliable biomarker for depression (Vammen et al., 2014).

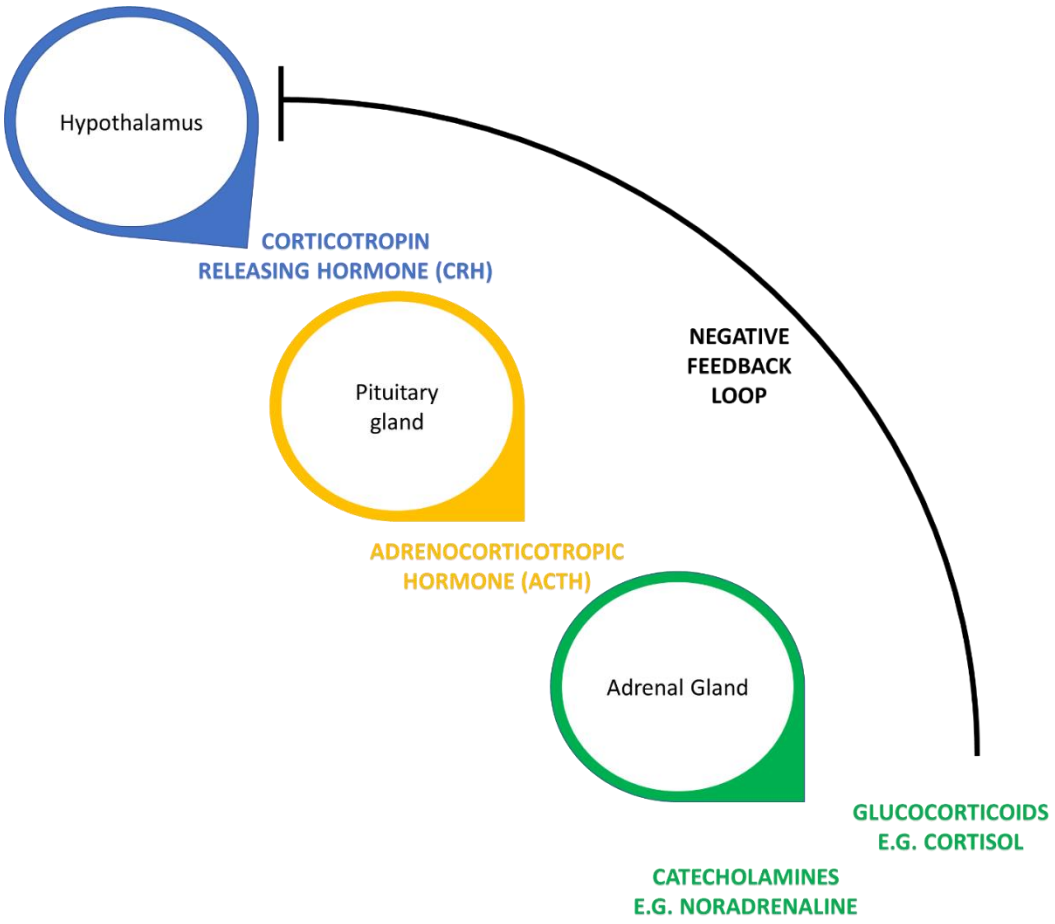

Figure 1.1. Diagram representing the hypothalamus-pituitary-adrenal (HPA) axis. The HPA axis is regulated by a negative feedback loop that that is initiated by the release of glucocorticoids that indirectly signal the hypothalamus to reduce the release of CRH.

Other neurotransmitters and neurotrophins

Brain-derived neurotrophic factor (BDNF), which is important for neuronal grown and survival, has also shown to be reduced in acute and chronic stress and is associated with higher cortisol levels (Belmaker & Agam, 2008). Reduced BDNF protein expression after chronic stress have been found in the dentate gyrus of the hippocampus which chronic antidepressant use has been shown to reverse (Grønli et al., 2006; Nair et al., 2007). However, inconsistent results of associations between BDNF levels and MDD has led to a lack of support for the BDNF hypothesis as a causal pathway for MDD (Groves, 2007).

Perhaps one of the most recent theories for MDD aetiology is the glutamate hypothesis. Glutamate is the main excitatory neurotransmitter in the brain and binds to the N-methyl-D-aspartate (NMDA) receptor. Its role in MDD was first noted in 1990 when NMDA receptor antagonists were found to have antidepressant-like effects (Trullas & Skolnick, 1990). Since then numerous studies have found that NMDA receptor antagonists exert fast and effective antidepressant action even in treatment resistant individuals (Gerhard, Wohleb, & Duman, 2016). Furthermore, ketamine (an NMDA receptor antagonist) has been found to upregulate synaptogenesis and reduce stress-induced neuronal atrophy (Duman & Li, 2012). Nevertheless, sustained use of ketamine can cause neuronal damage and, even at low doses, it can still exert psychotomimetic effects (Gerhard et al., 2016). Research into mimicking the effects of ketamine and other NMDA receptor antagonists without these adverse effects is ongoing (Gerhard et al., 2016).

Neuroinflammation

The cytokine hypothesis has also been proposed as an explanation for the aetiology of depression and the monoaminergic abnormalities. The presence of cytokines and inflammatory processes in MDD was first described in 1990 (Maes et al., 1990) but their association since then has become well established (Maes et al., 2009). Increased levels of inflammatory cytokines, have been found in the peripheral blood of MDD patients (Goldsmith, Rapaport, & Miller, 2016) and inhibition of proinflammatory cytokines has been shown to reduce depressive symptoms (Raison, Capuron, & Miller, 2006). Cytokines are known to induce sickness behaviours (such as fatigue and loss of appetite) which bear a notable resemblance to depressive symptoms; it is therefore hypothesised that depression could occur when the immune system is upregulated (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). Stress has also been linked to the regulation of inflammation; the HPA axis stimulates glucocorticoids to reduce transcription of proinflammatory cytokines and increase anti-inflammatory cytokines. However in chronic stress, desensitisation of the glucocorticoid receptors can occur which could intensify cytokine activity and in turn exaggerate chronic neuroinflammation (Y. K. Kim, Na, Myint, & Leonard, 2016). Furthermore, MDD has well established comorbidities with a variety of inflammatory diseases e.g.

cardiovascular diseases (Carney et al., 1988) and inflammatory bowel disease (Graff, Walker, & Bernstein, 2009). Nevertheless, C-reactive protein, a known inflammatory marker, has not been indicated as a causal risk factor for depression (Wium-Andersen, Orsted, & Nordestgaard, 2014). Moreover, a 2014 systematic review found a high level of heterogeneity in inflammation studies and could not reject the possibility of publication bias (Köhler et al., 2014). It is likely that inflammation occurs in a subgroup of MDD patients and therefore it has been suggested that future studies should focus on identifying patients that may benefit from anti-inflammatory intervention (Köhler, Krogh, Mors, & Benros, 2015).

Neurostructural mechanisms

Functional and structural brain abnormalities have been associated with MDD pathology. However, arguably the most widely studied neurostructural phenomenon in MDD causality is hippocampal neurogenesis. Adult neurogenesis (the generation of new neurons in the adult brain) is known to occur in two areas of the brain; the subventricular zone of the lateral ventricle and subgranular zone of the dentate gyrus in the hippocampus (Bond, Ming, & Song, 2015). It is regulated by both intrinsic and extrinsic mechanisms. Growth factors, neurotrophins (such as brain-derived neurotrophic factor; BDNF), neurotransmitters (e.g. glutamate) and sex hormones are among those intrinsic regulators to have been identified (Balu & Lucki, 2009). Other brain regions also have an involvement; the basolateral complex of the amygdala plays a regulatory role in hippocampal neurogenesis that in turn affects memory (Kirby et al., 2012). Genes are also key regulators of neurogenesis (Clark et al., 2011) and microglia (neuronal inflammatory agents) have been associated (De Lucia et al., 2016; Luo, Ikegaya, & Koyama, 2016).

The role of hippocampal neurogenesis in depression is still a controversial one and is commonly investigating by ablation of hippocampal neurogenesis in mice. High levels of stress have been demonstrated to decrease hippocampal neurogenesis in both animal and human models (Anacker, 2014) and animal models have demonstrated that antidepressants have the ability to increase production of new neurons (Eliwa, Belzung, & Surget, 2017). Furthermore, ablation of neurogenesis in the hippocampus inhibits antidepressant-induced remission and it has therefore been suggested that

neurogenesis mechanisms are essential for antidepressant effects (Surget et al., 2011). Diseases that are highly comorbid with MDD have also shown reductions in hippocampal neurogenesis: post-traumatic syndrome disorder (PTSD) (Besnard & Sahay, 2016), anxiety disorders (Schoenfeld & Cameron, 2015), schizophrenia (Duan et al., 2007) and Alzheimer's Disease (Kent & Mistlberger, 2017). Nonetheless, despite some evidence that increasing hippocampal neurogenesis reduces depressive-like behaviour (Hill, Sahay, & Hen, 2015), the majority of studies report that the ablation of new neuronal cells in the hippocampus is not associated with increased MDD and therefore not causative (Tanti & Belzung, 2013). Consequently, it has been hypothesised that antidepressant action may occur through increasing hippocampal neurogenesis causing an alteration in mood (Duman, Nakagawa, & Malberg, 2001) rather than targeting the cause of MDD. However, the exact role of neurogenesis in MDD and antidepressant response is still unknown.

Psychosocial factors (referring to the interrelationship of social factors and personality/behaviours) are also of significant importance as predictors of depression outcome. Associations have been found between the chronicity of depression and the HPA axis responsiveness to psychosocial stress (Booij, Bouma, de Jonge, Ormel, & Oldehinkel, 2013). In fact, when examining the impact of adolescent depression on onset of adult depression, the effect of adolescent depression was significantly reduced after accounting for psychosocial factors (McLeod, Horwood, & Fergusson, 2016). Personality factors can have a large impact on inter-individual response to stress (Lecic-Tosevski, Vukovic, & Stepanovic, 2011). Negative changes in psychosocial working environment have been shown to increase depressive symptoms indicating that psychosocial factors are important (J. Li et al., 2013). Additionally, psychosocial intervention has also been demonstrated to reduce postpartum depression, including nurse visits, peer telephone support and psychotherapy (Dennis & Dowswell, 2013). Psychosocial intervention strategies have also been shown to improve antidepressant adherence (Sirey, Bruce, & Kales, 2010) indicating their benefit in combination with pharmacotherapies.

The above research highlights the necessity for further research into the biological mechanisms involved in MDD. Both neurostructural and neurochemical mechanisms

have been implicated and it is possible that further investigation of these may elucidate more about MDD pathology. This thesis will explore structural neuroimaging, in order to determine more about neurostructural abnormalities, and antidepressant response, to determine whether differential response relates to variation in the underlying neurochemical processes. These subject areas are described in more detail below.

1.4. Regional Brain Volumes

Understanding of the processes in the brain has evolved considerably of the last few decades, but particularly with regards to emotion and cognition. This is of particular relevance as emotional dysregulation and cognitive deficits have been widely implicated in poor mental health outcome (Bradley et al., 2011; J. K. Trivedi, 2006). Both emotion and cognitive processes are predominantly controlled by complex interaction between subcortical and cortical structures (Heatherton & Wagner, 2011; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008) and, although defined as two separate entities, are thought to be highly interactive and interdependent (Storbeck & Clore, 2007). Specifically, emotional stimulus are generally preferentially remembered over neutral ones and they can therefore bias decision making and provide motivation whilst cognitive control can inhibit aggression and emotional regulation (Pourtois, Notebaert, & Verguts, 2012). Nevertheless, cognitive processes have been shown to deteriorate with age whereas emotion functioning remains unaffected (Ebner & Fischer, 2014). Emotional dysregulation is widely associated with MDD and has further been associated with cognitive inhibition of emotional material (Joormann & Gotlib, 2010) thereby implicating brain processes in MDD pathology. This thesis will focus on subcortical abnormalities associated with MDD in an aim to explicate the potential causality of structural differences in the disorder, however, it is worth mentioning that cortical structural differences have also been described in MDD. Determining these volumetric alterations could help determine individuals at risk and establish biological mechanisms. This section will give a brief overview of the known functions and associations of seven subcortical structures; the nucleus accumbens, caudate nucleus, putamen and pallidum (part of the basal ganglia), the hippocampus and amygdala (part of the limbic system) and the thalamus.

Subcortical volumes

Individual subcortical structures are known to be important in a variety of functions and are highly interactive between each other and other cortical regions. The nucleus accumbens has been traditionally associated with a role as a reward centre but has since been related to a variety of behaviours including learning, impulsivity and goal-orientated actions (Salgado & Kaplitt, 2015). The basal ganglia, composed of both the striatum (an interlinking complex of the caudate nucleus and the putamen) and the pallidum, has a well-established role in motor functions but has additionally been shown to have functions in time estimation, learning, emotions and cognitive function (Grahn, Parkinson, & Owen, 2008; Lanciego, Luquin, & Obeso, 2012). Moreover, the hippocampus has known functions in memory and cognition (Eichenbaum, 2004) whilst both the hippocampus and amygdala are known to play a role in emotions and memory (Phelps, 2004). Furthermore, the thalamus has been associated with cognitive function (Fama & Sullivan, 2015). Therefore, individual regions may contribute differentially to mental health outcomes.

Association with MDD

Neuroimaging findings in subcortical volumes in MDD are widely inconsistent. The largest single study to date, a UK Biobank study of 354 MDD cases and 803 controls, identified no significant differences in subcortical volumes, albeit the authors note their sample size as a limiting factor (Shen et al., 2017). In order to enhance sample size, a number of meta-analyses have been conducted. The ENIGMA Consortium conducted a large meta-analysis of subcortical structures finding that smaller hippocampal volume was associated with MDD (Cohen's $d = -0.14$, $P = 4.6 \times 10^{-4}$) and that this was driven by recurrent MDD (Cohen's $d = -0.17$, $P = 1.1 \times 10^{-5}$) (Schmaal et al., 2015). This study did not find any significant evidence for an association with any other subcortical volume after meta-analysis with 1,728 cases and 7,199 controls. A 2009 meta-analysis identified moderate reductions in the total volumes of the caudate nucleus (Cohen's $d = -0.31$, $P = 0.024$), putamen (Cohen's $d = -0.48$, $P = 0.003$) and hippocampus (Cohen's $d = -0.41$, $P < 0.001$) in an analysis of 2,418 MDD cases and 1,974 controls (Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009). Moreover, subcortical volumes, which are associated with age-related volumetric reductions, have been associated with accelerated aging in MDD; volume

reductions in the putamen were twice as large than in controls, with reductions of 16.7mm^3/yr in MDD cases (Sacchet, Camacho, Livermore, Thomas, & Gotlib, 2017). Nevertheless, in structural neuroimaging studies, reductions in hippocampus have been the most widely replicated finding (Arnone, McIntosh, Ebmeier, Munafò, & Anderson, 2012).

Association with Antidepressant response

Both structural and functional neuroimaging have demonstrated predictive potential in antidepressant response indicating their potential clinical utility (Fu, Steiner, & Costafreda, 2013). Specifically, the largest meta-analysis to date of hippocampal volumes in 374 MDD patients demonstrated smaller volumes were associated with antidepressant non-responders in comparison with responders (mean volume difference= 260mm^3, *P*= 0.002) (Colle et al., 2016). Furthermore, a study of 39 patients on fluoxetine found that larger whole brain volumes at trial initiation predicted successful response in 89% of cases and further theorised that structural abnormalities could specifically predict response to antidepressant treatment whilst functional changes could relate to response to cognitive-based therapies (CBT) (Costafreda, Chu, Ashburner, & Fu, 2009; Fu et al., 2013). Small sample sizes again severely limit the power to detect neuroimaging changes in treatment response studies (M. L. Phillips et al., 2015) and further research with larger samples are needed to produce reliable associations between antidepressants and subcortical volumes.

1.5. Antidepressants and MDD

Antidepressants are the main therapeutic option for individuals with MDD and are the third most commonly prescribed drug in the United States (US) (Mojtabai & Olfson, 2011). For the most part, they can be divided into seven main categories: monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs) tetracyclic antidepressants (TeCAs), serotonin antagonist and reuptake inhibitors (SARIs), selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NRIs) and serotonin and noradrenaline reuptake inhibitors (SNRIs). They were first approved for the treatment of major depressive disorder in the 1950s with SSRIs, now the most widely used antidepressant, introduced in the 1980s (López-Muñoz & Alamo, 2009). The latest antidepressant to be approved in Europe and the US was vortioxetine

in 2013 (Thase, Mahableshwarkar, Dragheim, Loft, & Vieta, 2016). However, although each of these antidepressant classes were targeted towards a particular mechanism, their actual mechanism of action is still largely unexplained (see below).

Class	Year of first approval	Intended Mechanism of Action	Examples
Monoamine oxidase inhibitors (MAOIs)	1952	MAOIs inhibits monoamine oxidase which is the enzyme responsible for the breakdown of monoamines. Monoamine oxidase is present as two isoenzymes MAO-A and MAO-B that differentially breakdown specific monoamines. MAOIs can reversibly or irreversibly inhibit both isoforms (Youdim, Edmondson, & Tipton, 2006).	Moclobemide Phenelzine Tranylcypromine
Tricyclic Antidepressants (TCAs)	1957	TCAs inhibit reuptake of both serotonin and noradrenaline and antagonise the histamine H1 receptor (Weber, Siddiqui, Wagstaff, & McCormack, 2010). Their chemical structure comprises of three rings.	Amitriptyline Dosulepin Lofepramine
Serotonin-noradrenaline reuptake inhibitors (SNRIs)	1971	SNRIs inhibit both the reuptake of serotonin and noradrenaline thereby increasing their availability at the synapse (M. Trivedi et al., 2008).	Duloxetine Venlafaxine
Tetracyclic antidepressants (TeCAs)	1974	TeCAs structure consists of four rings and have various differing effects amongst specific drugs. Mirtazapine (the most common TeCA prescribed for depression) increases serotonergic and noradrenergic neurotransmission via blocking α2 adrenergic receptors (Davis & Wilde, 1996).	Mirtazapine
Serotonin Antagonist and Reuptake Inhibitors (SARIs)	1981	SARIs block the 5-HT_{2A} receptors as well as the histamine H1 receptors and α1 adrenergic receptors (Stahl, 2009).	Trazodone Nafazodone
Selective Serotonin Reuptake Inhibitors (SSRIs)	1987	SSRIs act as selective antagonists at the serotonin transporter inhibiting reuptake at the synapse (Stahl, 1998).	Citalopram Fluoxetine Escitalopram
Noradrenaline Reuptake Inhibitors (NRIs)	1997	NRIs act as selective antagonists at the noradrenaline transporter in a similar action to SSRIs. They tend to have weak affinity for adrenergic and histaminergic receptors (Wong et al., 2000).	Reboxetine

1.2. Major classes of antidepressants. Each class of antidepressant also has its year of first approval, intended mechanism of action and examples of the major drugs.

Antidepressant response

Response to an antidepressant treatment is an improvement in symptoms whereas remission is defined as an improvement whereby the individual no longer reaches the criteria for major depressive disorder (Israel, 2006). Antidepressant response has been measured at about a third of individuals in remission after the first treatment trial (M. H. Trivedi et al., 2006) and half after the second treatment trial (A. J. Rush et al., 2006). Rush *et al.*, (2006) also found that response rates fell after each treatment step indicating that failure to receive remission was a risk for failure to respond the next treatment (response after 1st trial= 37%, 2nd trial= 31%, 3rd trial= 14%, 4th trial= 13%) (A. J. Rush et al., 2006). Currently the method for determining antidepressant prescription is trial and error with the low to modest response rates attributed to clinical subtypes of MDD (Domschke et al., 2016). The placebo effect (the simulation of a drug whereby improvement is due to personal expectations rather than the drug itself (Price, Finniss, & Benedetti, 2008)) is also commonly reported in antidepressants and widely debated with perhaps the most controversial study regarding this conducted by Kirsch *et al.*, (2008). The study documented the placebo effect in antidepressants, finding that it was accountable for response in all but the most severe cases of MDD, but this was concluded to be a decreased response to placebo not an increased response to medication (Kirsch et al., 2008). The study sparked immediate criticism with it being argued that response to antidepressants should be measured as the difference between drug and placebo, therefore the interpretation of the paper was in fact that drug response increases with depression severity (McAllister-Williams, 2008). Furthermore, a re-analysis of the data in 2011, pointed to several failings of this paper, including that both venlafaxine and paroxetine reduced depressive symptoms to a level that surpassed the thresholds set by the National Institute of Clinical Excellence (NICE) (Fountoulakis & Möller, 2011). Nonetheless, this subject is still widely debated (Bschor & Kilarski, 2016).

Mechanisms of action and non-response

Although the exact mechanism by which antidepressants exert an effect is unknown there is some evidence for their actions. Antidepressants were originally used in MDD due to their role in increasing monoamines, specifically serotonin. However,

monoaminergic increases occur quickly after taking an antidepressant whereas actual response (i.e. reduction in depressive symptoms) has been shown to take weeks (Andrade & Rao, 2010). Moreover, there is a vast amount of inconsistent evidence that exists for the role of serotonin, including no robust evidence that monoaminergic decreases are causal (Lacasse & Leo, 2005). It has therefore been proposed that antidepressant-induced reduction in depressive symptoms occurs via another mechanism. Antidepressants have been shown to cause increases in BDNF levels in almost every antidepressant class which have further been associated with antidepressant-induced behavioural changes in rodents (Castrén & Kojima, 2017). Furthermore, some antidepressants have been shown to reduce glutamatergic action by reducing the transmission and release of glutamate in the hippocampus and cortical regions (Musazzi, Treccani, Mallei, & Popoli, 2013). Higher inflammatory markers in peripheral blood has been shown to predict poor response to current treatments (Uher et al., 2014) and antidepressants have also been shown to affect levels of proinflammatory cytokines (Więdłocha et al., 2017), indicating a role of the inflammatory pathways. Antidepressants have also been shown to reverse stress-induced reduction in hippocampal neurogenesis (Warner-Schmidt & Duman, 2006) and, additionally, SSRIs have been indicated to enhance response to the environment which in turn facilitates drug response or non-response (Alboni et al., 2017). Nevertheless, no current explanations fully explicate antidepressant action and the pathways of response remain elusive. Explicating the mechanisms of action of antidepressants could provide an explanation for non-response, help identify more effective treatments and may also provide information on MDD causality.

Genetics

Familial concordance to differential antidepressant response has been shown (Franchini, Serretti, Gasperini, & Smeraldi, 1998) indicating there is a familial burden to drug response that could be attributed to genetic or shared environmental components. Genetic studies exploring differential responses have not identified any robust genetic marker. The main approach for measuring antidepressant response in MDD trials is a continuous measure of change in depressive symptoms from baseline, which are usually measured by the Montgomery-Asberg Depression Rating Scale

(MADRS) or the Hamilton Rating Depression Scale (HRDS). Initial studies of candidate genes (such as monoaminergic and neurotrophic polymorphisms) in antidepressant response produced poorly replicated results (Fabbri, Porcelli, & Serretti, 2014) and prompted a series of GWAS, that are generally considered the gold standard in genetic studies as they provide a hypothesis-free alternative. Nevertheless, no individual GWAS study or meta-analysis has yet produced any replicated genome-wide significant result (Biernacka et al., 2015; Cocchi et al., 2016; Garriock et al., 2010; GENDEP Investigators, MARS Investigators, & STAR*D Investigators, 2013; Ising et al., 2009; Q. S. Li, Tian, Seabrook, Drevets, & Narayan, 2016; Myung et al., 2015; Tansey et al., 2012; Uher et al., 2010). The largest clinically-measured study was a meta-analysis between NEWMEDS (Novel Methods leading to New Medications in Depression and Schizophrenia) and STAR*D (Sequenced Treatment Alternatives to Relieve Depression) which reached nearly 2,900 individuals. Antidepressant response was examined in multiple classes as well as an additional analysis exploring serotonergic and noradrenergic antidepressants, finding no significant genetic variants or pathways (Tansey et al., 2012). The major obstacle in these studies is sample size, as GWAS typically require tens of thousands of individuals to detect small effects. In order to enhance sample size, the 23andMe study applied self-report data to conduct the largest study to date on treatment resistance, providing data from 1,311 treatment resistant individuals and nearly 7,795 responder controls, but similarly, reported no significant associations. Nonetheless, self-report data is not generally considered as reliable as clinically-measured data and self-reported drug adherence has been shown to be inflated compared to other methodologies e.g. pharmacy-refill measures (Stirratt et al., 2015), indicating self-reported drug data may be subject to error.

Issues in antidepressant pharmacogenetic research

Pharmacogenetic studies and clinical trials generally provide small sample sizes. Randomised controlled trials (RCTs) are considered the optimum approach to study drug response as they give an unbiased comparison of placebo and drug. Nevertheless, they have met criticism due to the ethical considerations of distributing a placebo to a clinically ill population and are expensive to implement (Naudet, Maria, & Falissard,

2011). There are also few clinical trials in pharmacogenetics which have been attributed to lack of funding and small patient populations (Moaddeb & Haga, 2013). Moreover, it is only recently that pharmacogenomic (genome-wide) studies are beginning to be carried out in preference to candidate gene studies. Of note, antidepressant studies are also hindered by the placebo response. Whilst most clinical trials apply strict thresholds in order to decrease the number of placebo responders, they are estimated to account for 35-40% of all antidepressant responders (Furukawa et al., 2016).

Prediction of antidepressant response

Predicting individuals who may respond to an antidepressant could aid in understanding the mechanisms that drive response and non-response. Prediction models comprising of multiple clinical and demographic factors have been constructed for determining antidepressant remission or resistance. The most recent model was able to predict treatment resistance and remission to an accuracy of 0.74 and 0.85, respectively, by including as the predictors time between first and last depression episode, age and response to first antidepressant treatment, depression severity, education, occupation, and others. Nevertheless, none of these factors were significant independent predictors (Kautzky et al., 2017). In 2016, a prediction model found that depression severity, feeling restless, and reduced energy were the most significant predictors of non-remission whilst current employment, total years of education and loss of insight into one's depressive condition were the most significant predictors of remission. They reported that a model using 25 predictors of non-response gave an accuracy of 0.64 and were able to predict response in two out of three independent samples, suggesting that failure in the last sample may be due to their model being class-specific (Chekroud et al., 2016). Childhood trauma is the most replicated association with antidepressant non-response and, in a meta-analysis of 10 studies with over 3,000 participants, childhood maltreatment demonstrated a significant risk factor for lack of remission after an antidepressant (OR= 1.43, CI= 1.11–1.83) (Nanni, Uher, & Danese, 2012). In fact, it has been found that individuals who have experienced childhood trauma respond better to psychotherapies than antidepressants (Wald χ^2 = 6.89, *P*= 0.0087) (Nemeroff et al., 2003). Specifically, early-life trauma occurring

between four and seven years of age was found to be an important predictor of non-response (OR= 1.6, *P*= 0.034) (Williams, Debattista, Duchemin, Schatzberg, & Nemeroff, 2016). Therefore, it may be possible to utilise clinical and demographic factors in order to elucidate pathways to non-response.

Is treatment resistant depression a subgroup of MDD?

Research into polymorphisms in cytochrome P450s (metabolic enzymes) in antidepressant response have been inconsistent with positive findings generally reported in smaller samples (Wu-Chou, Liu, & Shen, 2016). Although further research is warranted in this area, it is also possible that that another mechanism is causing variation. Hudson and Pope (1990) theorised that differential antidepressant response could be utilised to determine overlapping disorders with the same underlying aetiology concluding that obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), panic disorder and bulimia likely shared aetiologies (Hudson & Pope, 1990). Furthermore, it has been previously speculated that the genetic architecture underlying antidepressant response actually demonstrates variability in MDD aetiology owing to evidence that the candidate genes associated with MDD have also been widely associated with variability in drug response in gene-environment studies (Keers & Uher, 2012). Nevertheless, results from candidate genes should be treated with caution as these have not yet been replicated genome-wide.

1.6. Project Summary and Aims

Summary

Despite recent success of GWAS, the aetiology of MDD remains unexplained. It is likely that the current MDD definition encompasses multiple aetiological subgroups and that dissecting these subgroups may be key to developing new medicines with higher efficacy. The modest heritability of MDD indicates that there are genetic underpinnings to the disorder and therefore genetic stratification may be of key value to identify subgroups. Given the vast literature on potential biological mechanisms of MDD, it is possible that current theories could provide a foundation for aetiological-based stratification. Two possible explanations of MDD could be explored further,

namely neurostructural (pertaining to brain volumes) and neurochemical theories (which could be examined by exploring antidepressant response).

Associations between regional brain volumes and MDD are frequently reported but results are often inconsistent. Nevertheless, the role of emotion and cognitive processes in MDD have been well documented and further explication of potential associations may be beneficial. Antidepressant response rates are low to modest and mechanisms behind non-response remain unknown. Genome-wide exploration of antidepressant response have yielded no replicated results, with small sample sizes likely the reason for this. To date, little is known about treatment resistant MDD and the mechanisms that drive it, therefore further studies utilising larger sample sizes are necessary.

Project aims

This thesis is divided into four main chapters that aim to investigate the above subject matter. Firstly, genetic stratification in psychiatric disorders and current methods that could be implemented to achieve this will be discussed. The aim of this chapter is to provide an overview of progress so far and outline whether genetic stratification in psychiatric disorders is achievable. Secondly, considering the inconsistent findings between both phenotypic and genetic associations of subcortical brain volumes and MDD, this chapter will explore their shared genetics using data from large consortia and genome-wide techniques in population- based cohorts. Potential genetic subgroups in MDD are also explored using subcortical volume genetic data. Thirdly, a genome-wide association study of antidepressant treatment resistance in MDD individuals will be examined in the largest study to date (excluding self-report studies). Narrow-sense heritability estimates and genetic correlations with associated traits will be investigated. Lastly, the impact and interrelationships of neuroticism, psychological resilience and coping styles in treatment resistance will be examined. Whilst increased neuroticism has been shown to be associated with treatment resistance, the role of resilience and coping style has not been previously studied. The next chapter will introduce genetic stratification and some of the methodologies that will be applied in this thesis.

Chapter 2. Genetic Stratification.

2.1. Background

In Chapter 1, current findings in MDD genetics were reviewed highlighting the high genetic overlap and substantial heterogeneity present in the disorder. Considering this and the poor to modest antidepressant response rates, it is possible that MDD is comprised of several different subgroups of disorders with varying causality. The previously reported genetic overlap between neuropsychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013) is especially interesting as it implies that current symptom-based diagnostic boundaries may encompass overlapping groups of similar aetiology. Nonetheless, no study to date has robustly stratified any neuropsychiatric disorder into aetiologically-defined groups, which may be essential for the construction of personalised therapies. This chapter will explore current methods for genetic stratification and how it can be achieved in neuropsychiatric disorders.

The content for this chapter has been summarised in a manuscript entitled “Towards Genetic Stratification of Neuropsychiatric Disorders” and is currently under revision at the Journal of Affective Disorders. As first author, I confirm that I composed and wrote the following paper.

2.2. Towards Genetic Stratification in Neuropsychiatric Disorders

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Number of words (main text)	3879
Number of references	91
Number of Tables	1
Number of Figures	2

2.2.1. Abstract

Background: Elucidating the genetic architecture underlying complex neuropsychiatric disorders has proven challenging, although it is known that these disorders have both genetic and environmental influences. Current psychiatric classification may group phenotypically similar individuals with diverse aetiologies and separate those with common disease mechanisms across diagnostic boundaries.

Results: In this current review, we discuss approaches for genetic stratification. Taking a broad quantitative approach to genomic analysis within and across diagnoses may aid establishment of mechanism-based stratification of psychiatric disorders where disease identification and future management is guided by genetic discovery. Intermediate phenotype approaches and machine learning techniques are currently being implemented to genetically stratify neuropsychiatric illness and may lead to revised classification and management.

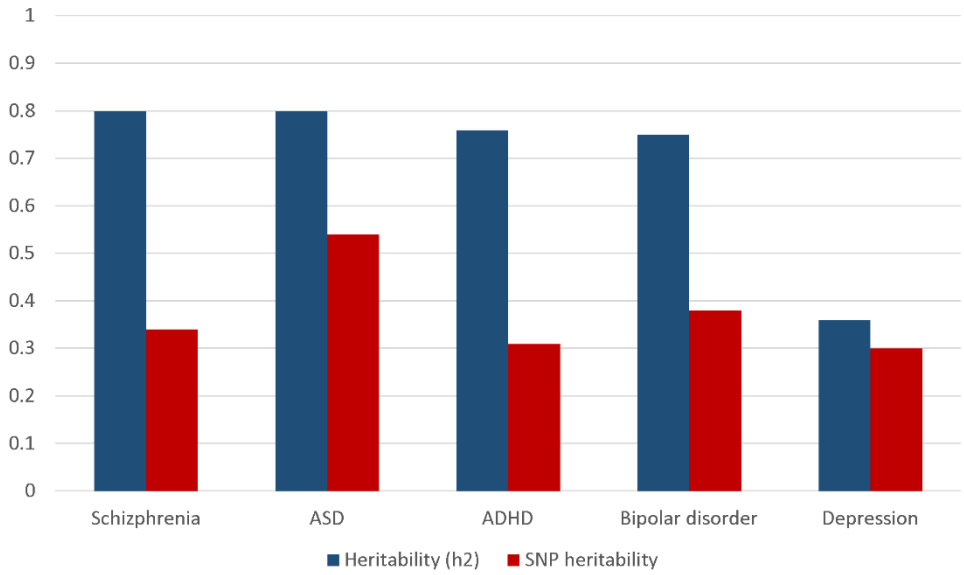
Limitations: Due to space constraints, this manuscript does not describe every genetic stratification technique and there may be others that can also be applied.

Conclusion: Utilisation of these techniques may stratify neuropsychiatric disorders into more homogenous groups that more accurately explain their aetiology.

2.2.2. Introduction

Elucidating the genetic and molecular mechanisms of neuropsychiatric disorders has proved to be very challenging, especially for common conditions, such as depression, that account for an expanding proportion of disability worldwide (P. Sullivan, Daly, & O'Donovan, 2012). Complex disorders (e.g. mood disorders, psychotic disorders, neurodegenerative disorders, eating disorders etc.) are caused by a combination of multiple genetic and environmental components (Hannan, 2013) and, in contrast to Mendelian disorders, have been less tractable to genetic research (Hirschhorn & Daly, 2005). This review will focus on common complex disorders examining how genetic subgroups can lead to aetiological-based stratification.

The genetic contribution to risk of a disorder is often measured by examining the disease risk in relatives of affected individuals. Heritability of neuropsychiatric disorders can be calculated using family-based and twin studies and cover a spectrum from 0.81 (P. F. Sullivan, Kendler, & Neale, 2003) for schizophrenia to 0.37 (P. Sullivan et al., 2000) for depression. Single nucleotide polymorphism (SNP) heritability estimates can be used in unrelated samples to measure the contribution of common variants but, in apparent contrast, these collectively explain a lower percentage of the total phenotypic variance than predicted from twin studies, 0.34 (International Schizophrenia Consortium et al., 2009) for schizophrenia and 0.21 for MDD (RipkeWray, et al., 2013). This 'missing heritability' can be explained in part by the aggregate effects of many common single nucleotide polymorphisms (SNPs) (J Yang, Lee, Goddard, & Visscher, 2011). Numerous studies have not only demonstrated the polygenic nature of complex psychiatric disorders (i.e. many common variants confer susceptibility in aggregate) but they have also shown that genetic liability can be shared across clinically separable disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; RipkeO'Dushlaine, et al., 2013). Explanations for the missing heritability problem include un-typed and poorly-imputed common variants, rare variants and interactions between genes (epistasis) and genes and environment (Wray & Maier, 2014). Failing to account for the effects of shared environment may also contribute to poorly estimated variant effect sizes (Manolio et al., 2009).



2.1. Heritability (h2) and SNP h2 of neuropsychiatric disorders. Narrow sense heritability figures and the SNP heritability estimated from GWAS for the five major neuropsychiatric disorders; schizophrenia h^2=0.80, SNP h^2=0.34 (International Schizophrenia Consortium et al., 2009), autism spectrum disorder h2=0.8, SNP h2=0.54 (Gaugler et al., 2014), ADHD h2=0.76, SNP h2=0.31(Saviouk et al., 2011) , bipolar disorder h2=0.75, SNP h2=0.38 (Lee, Wray, Goddard, & Visscher, 2011), and depression h2=0.36, SNP h2=0.3 (0.28-0.32) (Lubke et al., 2012). The gap between the h2 and estimated h2 from GWAS is what is termed the 'missing heritability' or unexplained heritability.

Phenotypic heterogeneity between individuals with the same disorder, as well as longitudinal variation within individuals over time, imposes a major obstacle to the study of the neuropsychiatric disorders (Hyman, 2007). The Diagnostic and Statistical Manual of Mental Disorders (DSM) is a diagnostic guide for psychiatrists that has met much criticism in recent literature due to its categorical distinction of psychiatric illnesses (Hyman, 2008; O'Donnell et al., 2014). Whilst it has provided a framework for clinical decision making and randomised trials, one of the major drawbacks has been the absence of any confirmatory biomarkers from the current diagnostic definitions. Many would argue that there has been an over-reliance on diagnosis through the identification of symptom clusters and a separation into 'ill' and 'well' based on an arbitrary thresholds (Hyman, 2007, 2008). It is possible, perhaps likely, that the current diagnostic categories in fact encompass different subgroups of patients

with similar symptoms, but differing aetiologies. This may also explain some of the missing heritability (Wray & Maier, 2014) as the variance explained by common SNPs can be attenuated by the inclusion of misclassified cases, especially in more common psychiatric disorders. Quantitative-trait approaches to genome analysis offers an alternative to qualitative disorder-based approaches. Significantly, these methods may allow us to re-evaluate the problems of psychiatric classification and distinguish more clinically tractable categories to improve patient treatment response and outcome.

This review will aim to give an overview of genetic stratification of neuropsychiatric disorders and the current techniques and will look at future directions for research in order to obtain clinically relevant genetic stratification.

2.2.3. What is genetic stratification and is it achievable?

Genetic stratification is the subgrouping of individuals based on similar underlying genetic architecture. In disease research, this can be useful to identify groups of individuals that could have the same disease aetiology. In many complex diseases, aetiologies have been difficult to identify prompting an exploration into innovative approaches to the problem. Genetic stratification could be key to developing personalised medication that more directly target to the mechanism of the disease.

Genetic *risk* stratification has been utilised in many different diseases ranging from common cancers to Alzheimer's disease. It is when individuals are considered high-risk for developing a disease if they carry a particular genetic variant, for instance, the BRCA1 and BRCA2 genes confer a very high risk of an individual developing breast or ovarian cancer (King, Marks, Mandell, & Group, 2003). If a high-risk variant is identified, genetic testing can identify these individuals and allow for early intervention e.g. early surgical interventions for BRCA1 or BRCA2 carriers (Finch et al., 2006; Kramer et al., 2005). However, in many complex diseases common variants, rare variants and environmental factors play a role and the presence or absence of any one genetic variant is a weak predictor of the presence or absence of disease. Genetic stratification aims to find subgroups of similar genetic architectures that are distinctly different from each other. It is currently being implemented in cancer research by The Cancer Genome Atlas (TCGA) Pan-cancer project (Weinstein et al., 2013). Cancers are generally classified based on the site of origin of the cancer, however, the results

of the project have indicated that there are genetic similarities in types of tumours that can span across different classes of cancers. This has encouraged a reassessment of current classification in cancer to include examination of tumour subtypes that could potentially lead to new therapeutics.

Whilst there has been much interest in this type of stratification in neuropsychiatry, subgroups have been difficult to identify. The Research Domain Criteria (RDoC) Project was set up in 2009 to identify more neurobiological subgroupings in mental illness by utilising seven different units of analysis including genetic analysis (Cuthbert & Insel, 2013; Insel et al., 2010). However, this research has yet to yield clinically useful subgroups, which is possibly attributable to its reliance on current diagnostic criteria (Kozak & Cuthbert, 2016) and very limited access to the tissue of interest compared to cancer. Nevertheless, new research and techniques have been making ground in psychiatric research. A recent paper examining resting state functional magnetic resonance imaging (fMRI) in depression patients identified 4 subgroups by hierarchical clustering that correlated to distinct symptom profiles (Drysdale et al., 2017). This review will examine machine learning techniques, such as this, that have been used to some success in genomic analysis as well as genomic techniques to examine the overlap and intermediate phenotypes in an aim to further dissect psychiatric disorders.

With increasing evidence suggesting a revision of the current diagnostic criteria and the known heritability and co-morbidity of neuropsychiatric disorders, they are ideal candidates for genetic stratification. In cancer genomic research, genetic stratification is already demonstrating great promise for identification of subgroups therefore demonstrating promise for its use in neuropsychiatry.

2.2.4. Genetic architecture of neuropsychiatric disorders

In order to genetically stratify a disorder, it is important to first understand its underlying architecture. Whilst there is a clear genetic contribution to neuropsychiatric disorders, their genetic architectures are incompletely understood (Gratten, Wray, Keller, & Visscher, 2014). The effects of individual alleles can be measured via their penetrance (the probability of a specific mutation eliciting a disease phenotype) and can also be involved in a vast network of interactions. Alleles at different loci may act

additively or interact with another (epistasis). All of these effects may also be moderated through their interaction with the environment, demonstrating the high complexity of this network.

This high polygenicity and clinical heterogeneity evident in neuropsychiatric disorders has fuelled the argument for stratification. Observed pleiotropy between disorders can both hinder and help explicate heterogeneity and can be explained as a genetic variant having a biological influence on multiple phenotypes (biological pleiotropy), traits being causally linked (mediated pleiotropy) or bias that can cause a false association, (spurious pleiotropy) (Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013). Dissecting this pleiotropy could be used to indicate genetically distinct subgroups of individuals with different underlying neuropsychiatric aetiologies. It is generally believed that the missing heritability can be accounted for by interacting genes, rare variants and gene-environment interaction (Wray & Maier, 2014). Many postulate that understanding the vast network of allelic variants and interactions could be an important component in understanding the mechanisms of the disorders (Caspi & Moffitt, 2006; Gratten et al., 2014). The identification of rare high-penetrance variants could be particularly valuable as the large biological effects they exert would present effective drug targets. Exome sequencing has provided an effective method of identifying rare variants and has described variants associated with Mendelian disorders (Ng et al., 2010) with some limited early success in complex diseases such as autism (Sanders et al., 2012).

Reports of gene-gene interactions have become increasingly frequent with demonstrations that their effects may exceed that of any single susceptibility gene (Kebir et al., 2014; Lai et al., 2015; Moore, 2003). Within psychiatry; evidence for epistatic interactions have been demonstrated in depression (Schott et al., 2014) and schizophrenia (KK Nicodemus et al., 2010a; KK Nicodemus et al., 2010b; KK Nicodemus et al., 2007) and there is emerging evidence that the inclusion of epistatic effects may improve prediction of psychiatric traits (KK Nicodemus et al., 2014). Environmental exposure to risk factors is also well-established in psychiatry and gene-environment interactions have attracted particular attention as a potential explanation for the inter-individual variability in disorder susceptibility (Duncan & Keller, 2011). Different environmental exposures have been shown to interact with different genes

to elicit the same psychiatric manifestation whereas it has also been shown that the same environment can interact with the same genes and lead to a different psychiatric indication (Klengel & Binder, 2013). Klengel and Binder (2013) therefore hypothesise that gene-environment interactions can be used to identify clinical psychiatric subtypes. The Environmental Genome Project (EGP) was set up to develop tools that can stratify individuals based on disease risk utilising integrative information on the genome and environmental exposures (Olden, Freudenberg, Dowd, & Shields, 2011). Attempts to identify these interactions have met some difficulty with the environment being both difficult to define and even more so to classify (Tsuang, Bar, Stone, & Faraone, 2004). With this complex underlying architecture, stratification presents a very challenging endeavour.

2.2.5. Genetic overlap and intermediate phenotypes in stratification

Exploring intermediate phenotypes and genetic overlap of related traits with disorders is a popular approach to examine the stratification problem. Intermediate phenotypes are phenotypes that are intermediate to a gene and outcome. In psychiatry these are often referred to as endophenotypes and definitions can differ between a mediation model (whereby the endophenotype is causal) and a liability index model (where the endophenotype may share characteristics) (Kendler & Neale, 2010). Endophenotypes or intermediate phenotypes have five criteria needed to confirm status which are: (1) the phenotype is associated with the illness, (2) it is heritable, (3) it is disease state-independent, (4) the endophenotype and illness co-segregate within families and (5) the endophenotype found in affected family members is found in a non-affected family member in a rate higher than in the general population (Gottesman & Gould, 2003). However, in the liability-index model the phenotype need not be state-independent, can be associated with a range of psychiatric diseases (not only with the illness of interest) and do not need to lie on the causal pathway (Flint, Timpson, & Munafò, 2014; Leuchter, Hunter, Krantz, & Cook, 2014). A method of testing some (but not all) of the criteria for intermediate phenotype status is to explore genetic overlap between a disorder and a potential intermediate phenotype. This would confirm a genetic association between the two phenotypes and indicate the shared genetic variation. Nevertheless, genetic overlap could be explained by biological pleiotropy, whereby a genetic variant induces both phenotypic outcomes, and therefore

investigates an indication for the liability-index model not a mediation model. Other psychiatric disorders have been indicated as potential intermediate phenotypes for each other. For instance, the Psychiatric Genomics Consortium (PGC) completed a cross-disorder GWAS across 5 psychiatric disorders; attention deficit-hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar disorder, depression and schizophrenia, and identified 4 loci that conferred risk across these disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). This provides further support for the need for a quantitative approach to investigate psychiatric disorders. Polygenic risk scoring (PRS), LD score regression and BUHMBOX (Breaking Up Heterogeneous Mixture Based On Cross-Locus Correlations) are all methods for examining genetic overlap.

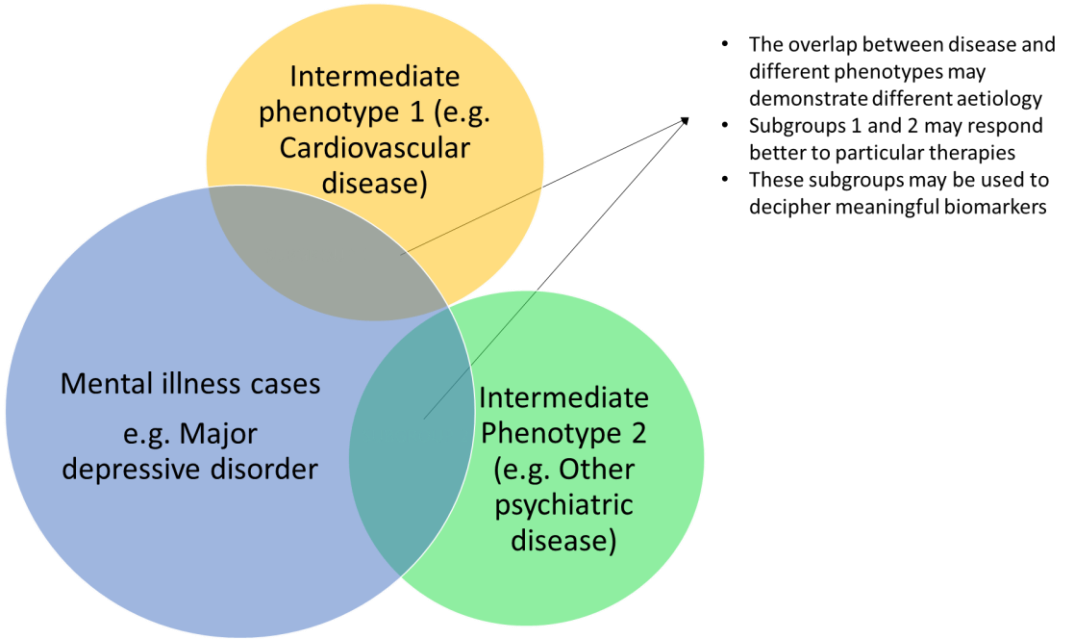


Figure 2.2. Illustration of the use of intermediate phenotypes in dissection of trait heterogeneity. Intermediate phenotypes can be used to isolate more homogenous groups in disease and aid personalised medicine.

PRS are utilised to infer a polygenic risk architecture and may also be used to measure the genetic overlap between disorders (i.e. pleiotropy). They utilise genetic markers' effect sizes estimated by GWAS on an independent training sample, such as those

provided by the PGC. Using these marker weights, a new 'discovery' sample is then genotyped and the product of each reference allele, multiplied by its effect size, is then determined across the genome. The 'risk score' is then calculated, for each individual in the discovery set, using the sum of the allele dosage effect size products (Dudbridge, 2013a; International Schizophrenia Consortium et al., 2009). PRS have been especially effective in neuropsychiatry where the availability of repeatable and objective biological correlates of disease is scarce. The first PRS implementation was provided in the International Schizophrenia Consortium (ISC) sample. It demonstrated that schizophrenia PRS, derived using marker weights from one GWAS, weakly predicted the diagnostic status of schizophrenia in a second independent case-control dataset and also weakly predicted a diagnosis of bipolar disorder (Craddock, O'Donovan, & Owen, 2005; International Schizophrenia Consortium et al., 2009). Similarly, depression PRS weakly predicted depression status explaining ~1% of the variance, and demonstrated genetic overlap with anxiety disorders (Demirkan et al., 2011). PRS has also been used to identify intermediate phenotypes; ADHD PRS (Martin, Hamshere, Stergiakouli, O'Donovan, & Thapar, 2014) and ASD PRS (Clarke, Lupton, et al., 2015) have both been associated with lower cognitive ability. Polygenic risk for schizophrenia has also been used to identify neural phenotypes such as prefrontal inefficiency; that may provide clues to the underlying neurobiological mechanisms of the disorder (Walton et al., 2014). PRS are now being used in an attempt to stratify neuropsychiatric disorders into more causally circumscribed diseases. In a recent paper examining the comorbidity between depression and migraine, Ligthart et al., (2014) found that pure migraine was genetically separate to migraine comorbid with depression on the basis of its associations with PRS. Furthermore, the authors concluded that migraine comorbid with depression was likely to have occurred as a consequence of depression (Ligthart et al., 2014). PRS can also be used to explore gene-environment interactions by investigating risk scores associated with specific risk factors (Nikolova, Ferrell, Manuck, & Hariri, 2011). For example, childhood trauma in individuals with a high polygenic risk for depression were found to be at greater risk of developing depression (Peyrot et al., 2014). Furthermore, PRS for obesity were found to have a greater effect on individuals with a lifetime diagnosis of depression (Clarke, Hall, et al., 2015).

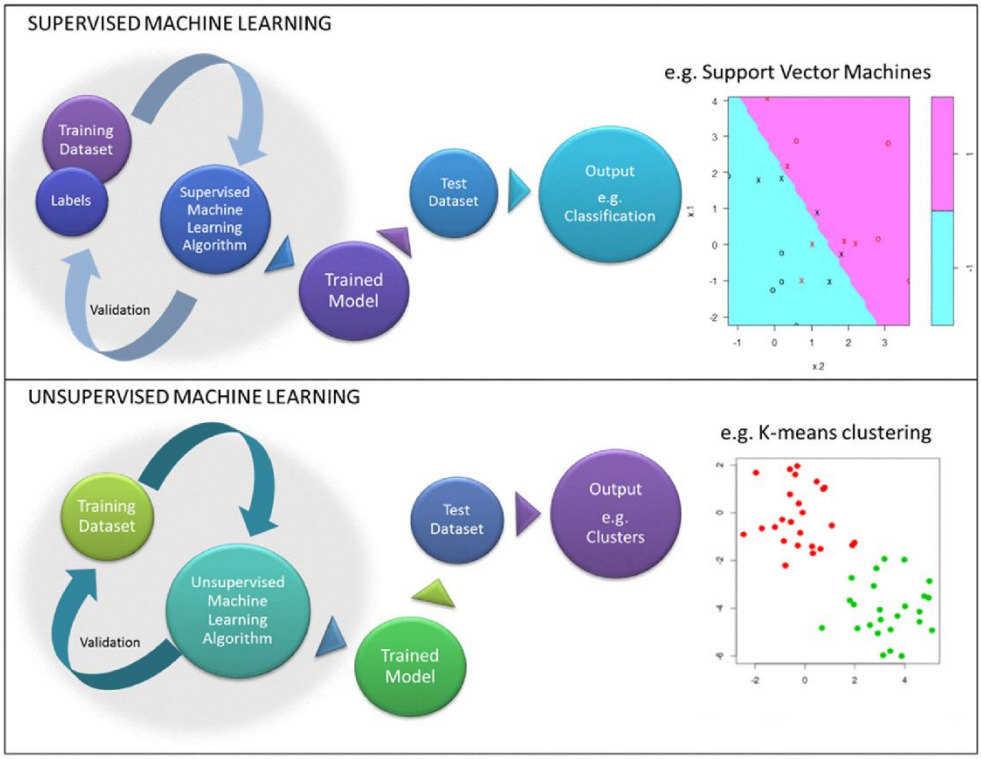
Linkage disequilibrium score regression (LDSC), a recent technique developed by Bulik-Sullivan et al. (2015), also estimates the proportion of variance in a trait due to common SNPs and additionally accounts for inflation due to polygenic signal. In LDSC, the chi-square score from GWAS summary statistics is regressed against the LD score, sum of LD r^2 between variants, and the intercept - 1 is then an estimator of the inflation. By utilising LD in this way, it aims to overcome the major problems LD causes in genome-wide studies. Simulation results of this study demonstrated that this tool was most powerful for polygenic traits, making it of particular interest in psychiatry. The score appears to work most efficiently when it is uncorrelated with the variance explained per SNP, although this is not always the case (BK Bulik-Sullivan et al., 2015a). A highlighted benefit of this technique is that is only requires genetic summary statistic data which are usually more widely accessible. Similarly to PRS, LDSC can also be adapted to measure genetic correlations between phenotypes by looking at the genetic covariance. It is a computationally efficient method that will not be biased by sample overlap, unlike genetic variance estimates generated by PRS. LDSC was applied to measure genetic correlation between psychiatric disorders and notably showed positive genetic correlation between ASD and educational attainment and near zero genetic correlation between Alzheimer's disease and psychiatric illness (B Bulik-Sullivan et al., 2015b). This further supports the theory that genetic exploration of these disorders could elucidate more about the nature of the illnesses and lead to improved stratification.

BUHMBOX is a recently developed technique, by Han et al. (2015), which can distinguish between clinical heterogeneity, defined as two genetically distinct subgroups, and true pleiotropy. It does this by utilising genome-wide data to examine risk alleles enriched in cases for two phenotypes. True pleiotropy would demonstrate enrichment across both phenotypes in cases but not controls whilst enrichment in only one of the phenotype cases and absent in the other demonstrates clinical heterogeneity. The method was utilised to examine autoimmune genetic sharing, concluding this was likely due to true pleiotropy, and was able to distinguish clinical heterogeneity between seronegative and seropositive rheumatoid arthritis. The method however does require large sample sizes and due to this was underpowered in an analysis on schizophrenia and depression (Han et al., 2015). It could provide a powerful technique, especially

with increasing sample sizes, in stratification of psychiatric traits into genetic subgroups.

2.2.6. Machine learning in genomic analysis of complex disorders

Machine learning defines an important set of tools that has gained much attention in recent biomedical literature. It is the application of an algorithm that is capable of learning from one dataset (usually called the training data) in order to instruct its application in another (the test dataset) in an attempt to demonstrate a validated relationship. It is therefore imperative for their utilisation that two independent datasets are used in order to test the validity of the model. Many machine learning algorithms are flexible to non-additive and non-linear genetic architectures and can provide an optimal fit to data containing these types of relationships. Supervised machine learning pertains to a method of fitting a model to a labelled dataset with predictors and response variables with an aim to accurately predict the response. Unsupervised learning refers to a method of fitting a model to data with no response variable (i.e. unlabelled data). Supervised methods are by far the most commonly utilised although there is a marked increase in the application of both methods within bioinformatics (Bhaskar, Hoyle, & Singh, 2006). A recent review summarises the progress machine learning has made in psychiatric disorders (Iniesta, Stahl, & McGuffin, 2016).



2.3. Supervised and unsupervised machine learning techniques. Supervised and unsupervised machine learning methods differ in that they contain labelled and unlabelled data, respectively. Models that overfit the training dataset can often not perform as well in a test dataset due to modelling noise from the training data. An example of each is shown; support vector machines are a supervised learning method for classification whereas k-means clustering is considered an unsupervised technique

The accessibility of genome-wide data imposes a heavy computational and multiple testing burden, with stringent p-value thresholds required to show statistical significance. Parametric statistical techniques can have severe limitations as they rely on making distributional assumptions (McKinney, Reif, Ritchie, & Moore, 2006) highlighting the need for parametric model enhancements and non-parametric alternatives. Owing to their low frequency in the general population, rare variants are difficult to identify, but a recent study demonstrated both supervised and unsupervised methods had increased power over standard statistical techniques in their detection (Lu, Austin, Bonner, Huang, & Cantor, 2014). In psychiatric research, random forest (RF), conditional inference forest (CIF), Monte Carlo Logic regression and gradient

descent boosting algorithms have also demonstrated potential in the determination of interactions; early studies have shown an association between polymorphic DISC, CIT and NDEL1 regions and NRG1, ERBB4 and AKT1 in schizophrenia case- control analyses (KK Nicodemus et al., 2010a; KK Nicodemus et al., 2010b).

Machine learning techniques can also be used to identify subgroups of individuals within current binary disease categories. These have been infrequently applied to psychiatry for genetic classification purposes, but have been effective in cancer genomics. For instance, in paediatric acute lymphoblastic leukaemia (ALL), hierarchical clustering and principle components analysis (PCA) were applied to a gene expression profiling and identified 7 subtypes of the disorder (Ross et al., 2003). In acute myeloid leukaemia (ALM) a bayesian machine learning algorithm (Bayesian Dirichlet processes) was applied to cytogenetic data, distinguishing 11 genetic subgroups of the disorder (Papaemmanuil et al., 2016). Utilising *k*-mean clustering and nonnegative matrix factorisation in transcriptomic data, a group were able to identify 6 subgroups of gliomas (A. Li et al., 2009). Hierarchical clustering also identified 5 subgroups of medulloblastoma in gene expression profiling (M. C. Thompson et al., 2006) and nonnegative matrix factorisation methods identified 3 genomic subgroups in copy number analysis using oligonucleotide-based array comparative genomic hybridisation in glioblastomas (Maher et al., 2006). Nevertheless, none of these studies have yet replicated their findings and it is therefore too early to speculate whether the groups identified are of clinical meaningfulness.

There is increasing evidence to support the ability of machine learning algorithms in high dimensional data (Chen & Ishwaran, 2012; J. McCarthy et al., 2004; Vert & Jacob, 2008). These tools can be particularly effective at prediction in high dimensions and have recently been applied to aid stratification in response to drug treatments. Support vector machine (SVM) methods were applied to a high dimensional dataset in order to predict response to Methylphenidate in ADHD. Employing genetics and neuroimaging results, amongst other information, SVM were able to distinguish the non-responder group with an 84.6% accuracy (J. W. Kim, Sharma, & Ryan, 2015). Similarly, RF and *k*-means clustering algorithms were applied to improve prediction of non-response to antidepressants and, utilising genomic and clinical predictors, were

able to improve prediction in an interaction-based model (Kautzky et al., 2015). Machine learning selection of predictors is also being applied to PRS techniques. In bipolar disorder a RF algorithm was applied to identify SNP predictors for construction of a PRS, demonstrating selection of 289 candidate SNPs enhanced the PRS sensitivity and specificity (Chuang & Kuo, 2017).

Machine Learning Techniques	**Supervised or Unsupervised**	**Function**	**Methodology**
Random Forest	Supervised	Classification, Regression	Decision trees are generated on a bootstrapped or sub-sampled training sample and the best-splitting predictors chosen from a set selected at random at each split. Each split in the tree considers one predictor and the following split made using the remaining sample, generating a recursively partitioned tree. Many trees are grown, generating a forest. The prediction error of the model can be tested on the left out group from the bootstrapped sample (Breiman, 2001; McKinney et al., 2006).
	Unsupervised	Clustering	In RF clustering, an RF dissimilarity measure, a numerical value of similarity between observations, is calculated. This measure is then utilised to generate clusters (Breiman, 2001; Shi & Horvath, 2006; Shi, Seligson, Belldegrun, Palotie, & Horvath, 2005).
Neural Networks	Supervised	Classification, Regression	Multiple layers of nodes connected by arcs with an initial input layer receiving the primary information and then connecting to varying levels of hidden layers before finally an output layer. Each input has a weight that is determined by a learning algorithm (Benitez, Castro, & Requena, 1997; McKinney et al., 2006).
	Unsupervised	Clustering	Involves assessing differences between weights and inputs in the network. The network with minimal difference can then adjust surrounding networks. This continues until clusters of networks are formed (Du, 2010).

Multidimensionality reduction (MDR)	Supervised	Classification	Reduction of the dimensionality of an N-dimensional model to a single dimensional model by grouping into multifactor classes. These multifactor classes could be low and high risk categories and are usually grouped utilising the Bayes classifier and 10-fold cross-validation (Cordell, 2009; Pattin et al., 2009; Ritchie, Hahn, & Moore, 2003).
Clustering	Unsupervised	Clustering	Clustering techniques are unsupervised methods utilised to detect patterns in datasets (Morris et al., 2011).
K-means clustering			Arguably the most widely used clustering algorithm, its function is to minimise the distance between data points and their closest centre to partition data into k sets. The mean is calculated determining the next point for the centre until the minimum distance is met (Kanungo et al., 2004).
Hierarchical clustering			Hierarchical clustering can be agglomerative (usually starting in individual clusters and merged at each step) or divisive (clusters are split at each step). Splitting or merging is completed on optimisation of a criterion e.g. sum of squares, nearest or farthest neighbour (Fraley & Raftery, 1998).
LASSO	Supervised	Classification	The LASSO performs variable selection and shrinkage. It is utilised in regression methods carried out with a penalty (usually termed L_1). In orthonormal designs, subset selection occurs using the largest coefficients contributing to the absolute value, setting some regression coefficients to zero whilst reducing others producing more easily interpretable results (Tibshirani, 1996).

2.3. Machine learning techniques. Table of various machine learning techniques and their methodologies.

Nonetheless, these techniques are not without their limitations. Due to the large datasets dealt with in machine learning, the algorithms can be susceptible to noise

within the data causing overfitting and poor predictive ability. Increasing dimensions within larger datasets also cause problems with inferring reliable conclusions, the so-called curse of dimensionality (de Ridder, de Ridder, & Reinders, 2013; Okser et al., 2014). Both of these limitations are often the reason for machine learning failure. Within genetic data, LD can also cause problems due to high genetic correlation (KK. Nicodemus, 2011; KK Nicodemus & Malley, 2009). Penalisation strategies such as the LASSO and ridge regression techniques are sometimes used in an attempt to prevent instability caused by LD (Okser et al., 2014). Moreover, it has been hypothesised that parametric techniques could be enhanced by combination with current machine learning algorithms (Moore, Asselbergs, & Williams, 2010). It is argued that even if machine learning provided a minimal increase in predictive power it would significantly improve clinical benefit (Okser, Pahikkala, & Aittokallio, 2013). Their use within psychiatry would certainly aid in stratification but these limitations are not without merit and therefore caution is warranted. Techniques should be comprehensively evaluated before application and results should be independently replicated before any clinical conclusions are drawn.

2.2.7. Discussion

Important genetic influences on the risk of neuropsychiatric disorders have been established beyond reasonable doubt and these findings lay the foundation for future genetic stratification. Nevertheless, the current classification system within psychiatry is sub-optimal and unable to account for both phenotypic and genotypic overlap between disorders, as well as the qualitative distinction of 'ill' and 'well' individuals. To further understand the genetic architecture behind these disorders it may be beneficial and more parsimonious to examine the quantitative traits underlying clinical disorders, without imposing a somewhat arbitrary threshold. Consequently, approaching disease stratification through the genetic architectures underlying quantitative traits could assist in the identification of more causally circumscribed disease entities with greater predictive utility.

Genomic analysis in complex psychiatric disorders is burdened with high heterogeneity and polygenicity. With rare and common genetic variants, epistatic and environmental interaction, the architecture behind these disorders is exceptionally complex making their genetic dissection especially challenging. Examining genetic overlap with related traits and intermediate phenotypes can be examined by PRS, LDSC and BUHMBOX, but all of these techniques are reliant on large sample sizes for increased power. All the genetic and environmental interactive possibilities lead to high dimensional data which traditional parametric technique are typically not equipped to deal with. Machine learning provides a potential alternative as it can be tailored to cope with high dimensions. Its growing popularity in genomic analysis and promising results, especially in cancer genomics, has supported the contention that it could be a useful tool in complex traits.

It is generally considered that we are entering the age of 'big data' whereby researchers have access to multiple large datasets from differing sources, exerting high demands on current computational resources. A lack of confidence in the DSM definitions of psychiatric disorders presents additional obstacles to analysis that are imposed by the thresholding of clinical syndromes as binary traits. It is likely that the use of quantitative traits in combination with binary clinically-diagnosed phenotypes offer a more tractable and potentially effective approach to future research. A combination of

the above techniques could be effective approaches to tackling the classification problem. Through the dissection of their genetic architectures perhaps we can utilise large datasets of clinical and genomic data to investigate and validate candidate disease taxonomies and match these to case by case clinical management.

Conflicts of Interest

The authors declare no conflicts of interest.

Role of the funding source

The funding sources had no role in study design, in the collection and analysis of data, in the writing of the report, and in the decision to submit the article for publication

Acknowledgements

AMM is supported by the Wellcome Trust 104036/Z/14/Z (STRADL, Stratifying Resilience and Depression Longitudinally) and Sackler Foundation.

2.3. Chapter Conclusion

This chapter summarises the current techniques for genetic stratification and how they can be applied within neuropsychiatry. Current advances in cancer, demonstrate the utility of genetic stratification as a tool to reduce heterogeneity. Two main approaches were discussed; the intermediate phenotype approach and machine learning techniques. Intermediate phenotypes (the study of a phenotype that could mediate the association between gene and outcome) have been studied in some detail in neuropsychiatric diseases but machine learning techniques have been less frequently applied. They are more commonly employed in cancer research but represents a useful future tool for stratification of psychiatry in large datasets.

Genetic overlap between MDD and other diseases/disorders has previously been documented (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Ji et al., 2016) and lends support to the hypothesis of genetic subgroups. Identifying such subgroups could aid in the generation of personalised therapies. Genetic overlap also indicates a possible presence of intermediate phenotypes which could be used to identify subgroups and possibly biomarkers, given that the most useful biomarkers will be those with intermediate proximity between gene and outcome (Leuchter et al., 2014). To identify phenotype as an intermediate, it must meet several criteria and so genetic overlap is only an indication. However, it is an effective method to indicate the presence of associated phenotypes that represent candidates for intermediate phenotypes. In this thesis, the intermediate phenotype approach will be applied to two areas of active research in MDD; regional brain volumes and antidepressant treatment resistance.

Chapter 3. Regional Brain Volume and MDD.

3.1. Background

Differences in regional brain volumes in individuals affected by MDD compared to controls have been described previously but results have been inconsistent. A general summary of current structural findings was given in Chapter 1. Here, I will comment on the genetic associations that have been found between regional brain volumes and MDD and describe why a genome-wide approach is needed.

Much like MDD, subcortical volumes are determined by both genetic and environmental components (Wen et al., 2016) and further have been found to be influenced by common variants (Hibar et al., 2015). Given that structural abnormalities have previously been associated with a higher risk of depression (Koolschijn et al., 2009; Schmaal et al., 2015), brain volume could be examined as an intermediate phenotype between genes and MDD that encode the genetic liability and causally impact disease risk. In fact a number of studies have indicated brain volumes and imaging phenotypes to have role as intermediate phenotypes in psychiatric disease (Honea et al., 2008; Meyer-Lindenberg & Weinberger, 2006). Candidate genes have been the most commonly adopted approach to do investigate genetic overlap and monoaminergic polymorphisms have been most widely examined. For example, recent studies exploring serotonergic polymorphisms have demonstrated moderate associations with amygdala volume in a sample of 417 healthy controls and right pallidum volume in a sample of 42 MDD cases and 15 healthy controls (Jaworska, MacMaster, Foster, & Ramasubbu, 2016; J. Li et al., 2015). Nevertheless, results from such studies have been largely inconsistent, reporting both significant associations and lack thereof (Won & Ham, 2016). This inconsistency may be due to an increased likelihood of reporting false positives due to the bias introduced by a hypothesis-driven selection of a candidate gene that may or may not be associated with the outcome (especially if the candidate gene has not been replicated genome-wide) and low sample sizes in neuroimaging studies (Scharinger, Rabl, Pezawas, & Kasper,

2011; P. M. Thompson et al., 2014). This advocates the use of genome-wide approaches such as genome-wide association studies (GWAS) (which are hypothesis free) and subsequently linkage disequilibrium score regression (LDSC) and polygenic risk scoring (PRS) techniques. More recently, a PRS analysis was applied to a sample of 938 individuals and the authors reported that PRS of MDD, schizophrenia and bipolar disorder were not associated with subcortical volumes (Reus et al., 2017). However, a sample of 938 individuals is likely underpowered for a PRS analysis (Dudbridge, 2013b). In order to apply genome-wide techniques to examine genetic overlap between MDD and regional brain volumes and indicate their potential role as intermediate phenotypes, much larger samples are needed. Recent endeavours have seen considerable sample increases led by efforts from the ENIGMA (Enhancing NeuroImaging Genetics through Meta-Analysis) consortium, using meta-analysed data from numerous existing neuroimaging cohorts, and population-based cohorts such as UK Biobank, that recently released magnetic resonance imaging (MRI) data on 100,000 individuals. In fact, in 2015, the ENIGMA consortium released the first ever GWAS of regional brain volumes (Hibar et al., 2015). Equipped with these larger samples and more sufficiently powered GWAS, genome-wide approaches could be applied in order to determine whether genetic overlap is present and regional brain volumes represent potential intermediate phenotypes between genes and MDD outcome.

Using the summary data from the recent ENIGMA GWAS, this following study will use LDSC and PRS techniques to determine genetic overlap between regional brain volumes and MDD. Additionally, Mendelian Randomisation (MR) and BUHMBOX were applied to test for directional association and determine the presence of MDD subgroups enriched for alleles associated with regional brain volumes. The content for this chapter has been summarised in a manuscript entitled “Do regional brain volumes and major depressive disorder share genetic architecture? A study of Generation Scotland (n=19 762), UK Biobank (n=24 048) and the English Longitudinal Study of Ageing (n=5766)” which has been published in the journal ‘Translational Psychiatry’ (Wigmore et al., 2017). I confirm, as the first author, that I carried out the analysis of the data and wrote the following paper.

OPEN

Citation: Transl Psychiatry (2017) 7, e1205; doi:10.1038/tp.2017.148

www.nature.com/tp

ORIGINAL ARTICLE

Do regional brain volumes and major depressive disorder share genetic architecture? A study of Generation Scotland ($n = 19\,762$), UK Biobank ($n = 24\,048$) and the English Longitudinal Study of Ageing ($n = 5766$)

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Major depressive disorder (MDD) is a heritable and highly debilitating condition. It is commonly associated with subcortical volumetric abnormalities, the most replicated of these being reduced hippocampal volume. Using the most recent published data from Enhancing Neuroimaging Genetics through Meta-analysis (ENIGMA) consortium's genome-wide association study of regional brain volume, we sought to test whether there is shared genetic architecture between seven subcortical brain volumes and intracranial volume (ICV) and MDD. We explored this using linkage disequilibrium score regression, polygenic risk scoring (PRS) techniques, Mendelian randomisation (MR) analysis and BUHMBOX. Utilising summary statistics from ENIGMA and Psychiatric Genomics Consortium, we demonstrated that hippocampal volume was positively genetically correlated with MDD ($r_G = 0.46$, $P = 0.02$), although this did not survive multiple comparison testing. None of the other six brain regions studied were genetically correlated and amygdala volume heritability was too low for analysis. Using PRS analysis, no regional volumetric PRS demonstrated a significant association with MDD or recurrent MDD. MR analysis in hippocampal volume and MDD identified no causal association, however, BUHMBOX analysis identified genetic subgrouping in GS:SFHS MDD cases only ($P = 0.00281$). In this study, we provide some evidence that hippocampal volume and MDD may share genetic architecture in a subgroup of individuals, albeit the genetic correlation did not survive multiple testing correction and genetic subgroup heterogeneity was not replicated. In contrast, we found no evidence to support a shared genetic architecture between MDD and other regional subcortical volumes or ICV.

Translational Psychiatry (2017) **7,** e1205; doi:10.1038/tp.2017.148; published online 15 August 2017

INTRODUCTION

Major depressive disorder (MDD) is a debilitating condition that accounts for a large proportion of disease burden world-wide.[1] It is a complex disorder that is influenced by both genetic and environmental factors with a heritability of ~37% estimated from twin studies.[2] Two recent genome-wide association studies (GWAS) identified two loci in MDD[3] and 15 loci in self-reported depression[4] of genome-wide significance. Nevertheless, the majority of MDD's heritability is unaccounted for by currently identified variants and the mechanisms leading from gene to clinical phenotype remain elusive.

Reports of lower brain volumes in cross-sectional studies are common in MDD, but small sample sizes have potentially contributed to poorly replicated results. Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) completed a large MDD case–control meta-analysis of subcortical volumes ($n = 8927$) demonstrating a significant association between MDD and reduced hippocampal volume (Cohen's $d = -0.20$).[5] Numerous other studies have also demonstrated a link between hippocampal reduction and MDD, and it is one of the most robustly associated brain regions.[6] Other brain regions have shown limited and sometimes contradictory evidence for association with MDD. Smaller amygdala volume has been associated with depressive symptoms[7,8] and MDD status,[9] however larger amygdala volume has also been associated with the disorder.[10] A 2013 meta-analysis concluded that, as well as hippocampus, smaller putamen and thalamus volumes were associated with late life MDD, although fewer studies have examined these regions.[11] In addition, smaller caudate nucleus volumes have also been associated with MDD in a meta-analysis.[12] The nucleus accumbens has not been widely associated with MDD status but a smaller volume has been implicated in the lethality of suicidal acts within mood disorder sufferers.[13] Pallidum volume and intracranial volume (ICV) have not been associated with MDD in any meta-analysis to date, as far as we are aware.

Subcortical structural volumes are known to be influenced by both genetic and environmental factors and have been demonstrated to be moderately to highly heritable ranging from 0.44 to 0.88.[14] The previously reported lower brain volumes in MDD and the relatively high heritability of these structures means they

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Received 8 December 2016; revised 8 May 2017; accepted 7 June 2017

could be of interest as an intermediate phenotype.[14] Overlap between genes involved in MDD and subcortical regions have been explored previously. The majority of studies have focused on candidate genes, such as the serotonin transporter (5-HTTLPR), and findings have often been contradictory.[15] As the success of a candidate gene study is reliant on the correct gene being chosen, GWAS studies are often considered to be a less biased and more reliable approach.[16] GWAS of regional brain volumes has recently been completed by the ENIGMA Consortium,[17] providing an important opportunity to examine the genetic overlap between subcortical brain volumes and ICV with MDD. Indications of covariation could potentially identify the risk conferring loci involved in MDD as well as the underlying mechanisms.

In this current study, we examine whether the genetic architecture of MDD is shared with multiple subcortical brain regions and ICV. We employed four techniques; the first, linkage disequilibrium (LD) score regression,[18,19] estimates the genetic correlation between these traits using GWAS summary statistics from the ENIGMA consortium and Psychiatric Genomics Consortium (PGC). The second method, polygenic risk scoring (PRS),[20] utilises ENIGMA summary statistics to generate individual level polygenic profile scores of each brain region's volume. We then calculated the association of PRS (a) with their own volume in UK Biobank and (b) with MDD status in three population-based cohorts; Generation Scotland: Scottish Family Health Study (GS:SFHS), English Longitudinal Study of Ageing (ELSA) and UK Biobank and (c) with recurrent MDD, MDD episodes, MDD duration and age of onset in GS:SFHS and UK Biobank. Both (b) and (c) analyses were adjusted for confounds on an individual subject level and then combined in a meta-analysis. Third, we used the Mendelian randomisation method[21] to examine a directional causal relationship between the regional volumes and MDD, utilising the GWAS significant loci as genetic instruments. Lastly, we used a new software package BUHMBOX[22] to test for the presence of genetic subgroup heterogeneity.

MATERIALS AND METHODS

Cohort descriptions and genotyping

Generation Scotland: Scottish Family Health Study. GS:SFHS is a family-based cohort with phenotypic data for 24 080 participants (mean age = 47.6, s.d. = 15.4) of which 20 032 had genotype data. Individuals were eligible if they had one first-degree relative willing to partake in the study. Further details on the recruitment for this cohort are available in the Supplementary Materials and have been described previously.[23] Diagnosis of MDD was made using the structured clinical interview for DSM-IV disorders (SCID) for those individuals that screened positive during interview questions ($n = 19\,762$, cases = 2643).[24] Individuals with bipolar disorder ($n = 76$) were excluded from this study. Information on MDD episodes and age of onset was also included in the SCID and therefore recurrent MDD and duration of MDD could be inferred (further details are given in the Supplementary Materials).

Details of the DNA extraction for GS:SFHS have been previously described.[25] Genotyping was completed at the Wellcome Trust Clinical Research Facility Genetics Core, Edinburgh (www.wtcrf.ed.ac.uk) using the Illumina HumanOmniExpressExome -8v1.0 Beadchip (San Diego, CA, USA) and Infinium chemistry[26] and processed using GenomeStudio Analysis Software v2011.1. Quality Control (QC) utilised the following inclusion thresholds; missingness per individual $< 1\%$, missingness per single-nucleotide polymorphism (SNP) $< 1\%$, Hardy–Weinberg Equilibrium (HWE) *P*-value $> 1 \times 10^{-6}$, minor allele frequency (MAF) $> 1\%$. There were 556 705 SNPs and 19 994 individuals that passed QC criteria.

UK Biobank. UK Biobank is an open resource cohort with phenotypic data for 502 664 (mean age = 56.5, s.d. = 8.1) between the ages of 40–69 recruited within the United Kingdom between 2006 and 2010, with genotype data available for 152 734 participants. Our study was conducted under UK Biobank application 4844. Study design and recruitment has been described previously[27] but, in brief, participants were asked to complete a touchscreen questionnaire and additional data were collected by nurse interview. MDD status was based upon putative MDD phenotype defined by Smith *et al.*[28] ($n = 24\,048$). Participants with mild depressive symptoms were removed based on this definition and self-reported bipolar disorder participants ($n = 1211$) were excluded. Information on MDD episodes and age of onset was also available, therefore recurrent MDD and MDD duration was inferred (further details are given in the Supplementary Materials). Subcortical volumes for nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen and thalamus were measured by T1-weighted structural imaging. The UK Biobank imaging protocol has been described elsewhere (http://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id = 1977). The mean of the sum of left and right volume was taken for each subcortical region. ICV was generated by the sum of white matter, grey matter and ventricular cerebrospinal fluid volumes. Imaging data for the eight structures were available for 4446 participants, of which 968 had genetic data available.

Genotyping was completed utilising two Affymetrix arrays (Santa Clara, CA, USA); BiLEVE ($n = 49\,979$) and the UK Biobank Axiom ($n = 102\,750$). Details have been described previously.[29] Initial genotyping QC was performed by UK Biobank.[30] Additional filtering was then applied to participants with poor heterozygosity or missingness, QC failure, non-British White ancestry, gender mismatch, genotype missingness $< 2\%$, and relatedness within UK Biobank and to the GS:SFHS sample ($r > 0.0442$, $n = 35\,752$) and ELSA sample (in the meta-analysis with all three cohorts). SNPs inclusion criteria were HWE $P > 1 \times 10^{-6}$ and MAF $> 1\%$. There were 731 536 SNPs and 152 735 individuals that passed QC criteria.

English Longitudinal Study of Ageing. ELSA is a prospective cohort study of health and ageing collected in 2002 with six follow-up waves taken at 2-year intervals. At wave 1 (baseline), phenotypic data were available for 12 003 (mean age = 63.9, s.d. = 10.7) and genotypic data available for 7452 participants. Details of this cohort have been described previously[31] and further information is available in the Supplementary Materials. MDD status in this study was defined using a shortened form of the Centre of Epidemiological Studies—Depression scale (CES-D scale) (completed by 5752 participants with genomic data). This consisted of 8 questions, rather than the original 20, with a 'no'/'yes' response that was converted to a binary 0/1, respectively, although positive questions, that is, 'During the past week, were you happy?', were scored in reverse; 0 being 'yes' and 1 being 'no'. After summing the scores, a dummy variable of MDD status was classified as those with a score of 4 or above, as in previous studies.[32] Self-reported 'manic depressive' ($n = 41$) individuals were excluded.

Genotyping was completed in 2013/14 on 7452 participants on the Illumina Omni 2.5–8 chip and QC and removal of related individuals ($r \geq 0.2$, $n = 109$) was completed at the University College London Genetics Institute. Further QC was implemented using the same inclusion thresholds as used for GS:SFHS; SNP inclusion criteria were HWE $P > 1 \times 10^{-6}$ and MAF $> 1\%$ and exclusion of related individuals ($r >= 0.2$, $n = 109$). There were > 1.3 million SNPs and 7230 individuals that passed QC criteria.

LD score regression

Genetic correlation of subcortical structures and ICV with MDD were measured using the LD score regression technique.[18,19] In brief, this technique utilises GWAS summary statistics to estimate the SNP-based heritability of a trait and genetic correlation between traits, in this study we used summary data from ENIGMA and PGC. SNPs inclusion criteria were INFO > 0.9 and MAF $> \%1$ (further details in Supplementary Materials).

Summary statistics for the regional brain volume GWAS completed by ENIGMA were downloaded from http://enigma.ini.usc.edu/research/download-enigma-gwas-results/. The GWAS was completed using 11 840 participants for eight MRI volumetric measures; nucleus accumbens, amygdala, caudate nucleus, hippocampus, pallidum, putamen, thalamus and ICV.[17]

Summary statistics for the MDD GWAS completed by the MDD Working Group of the PGC were downloaded from http://www.med.unc.edu/pgc/downloads. The study examined 9238 MDD cases and 8039 controls.[33]

Polygenic risk scoring

Construction of PRS was completed in PLINK software.[34] PRS utilise effect sizes from GWAS summary statistics to construct an additive individual genetic scores in a population.[20] Summary statistics were taken from the ENIGMA GWAS[17] (details above) to construct weighted PRS using five *P*-value thresholds: 0.01, 0.05, 0.1, 0.5 and 1, after SNPs underwent clumped-based pruning ($r^2 = 0.25$, 300 kb window). All five thresholds are

reported in models of subcortical volume and ICV PRS predicting their respective volume in UK Biobank and the best predictive threshold was carried forward into models associating MDD status with each subcortical volume and ICV in all three cohorts. The *P*-value thresholds carried forward were; nucleus accumbens: 0.01, amygdala: 0.1, caudate nucleus: 0.5, hippocampus: 0.01, ICV: 0.5, pallidum: 0.5, putamen: 0.1 and thalamus: 0.05. Scores for GS:SFHS, UK Biobank and ELSA were computed on the raw genotypes.

Statistical analysis

Association between regional brain volume PRS and its respective volume. Models predicting regional brain volumes in UK Biobank were conducted using linear regression in R version 3.2.3 (www.r-project.org). Models were adjusted for age, sex and the first 15 principal components (PCs) as well as for ICV (excepting ICV itself).

Association between regional brain volume PRS and MDD. Mixed linear model analyses were completed in ASReml-R (http://www.vsni.co.uk/software/asreml/) for GS:SFHS with MDD status. Mixed linear modelling was utilised to account for the family structure in GS:SFHS. MDD status was fitted as the dependent variable and volume PRS fitted as the independent variable. The model was adjusted for age and sex with the first four PCs fitted to control for population stratification. An additive relationship matrix (expected relatedness derived from pedigree information) was fitted as a random effect to account for the family structure in GS:SFHS. Wald's conditional F-test was used to calculate *P*-values for all fixed effects and the variance explained was calculated by division of the difference in the sum of residual variance and additive genetic effect in the null model (without PRS) with the full model (with PRS). To adjust for the use of linear-mixed regression models being applied to a binary dependent variable in a structured data set, the fixed effects and standard errors from the linear model were transformed utilising a Taylor series approximation[35] from the linear scale to the liability scale (Supplementary Materials).

In unrelated samples (UK Biobank and ELSA) logistic regression utilising generalised linear models in R version 3.2.3 (www.r-project.org) was used to test the degree of association between MDD and PRS of subcortical volumes and ICV. Models were adjusted for age, sex and the first 15 PCs (in UK Biobank) and first 4 PCs (in ELSA) to control for varying levels of population stratification present in the samples.

Association between hippocampus volume PRS and MDD traits. As hippocampal volumetric differences have been more closely associated with recurrent MDD and early illness onset,[5,36] hippocampus PRS regression analyses were also run with recurrent MDD, number of episodes, MDD duration and age of onset as dependent variables (for further details see Supplementary Materials). In GS:SFHS these were run utilising mixed linear model analysis (as above) to account for the family structure. As recurrent MDD is a binary trait, this was transformed from the linear to liability scale using the Taylor series approximation[35] (as above). For testing association in unrelated samples, logistic regression models were used for binary traits (recurrent MDD) and linear regression for quantitative traits (number of episodes, MDD duration and age of onset). Models were adjusted for age, sex and the first 15 PCs to control for population stratification. These data were not available for ELSA therefore this was run in UK Biobank only.

Meta-analysis. In order to increase power, fixed effect meta-analysis, weighted by standard error of the beta values relating PRS scores to MDD was carried out using the 'meta' package (version 4.3-2)[37] in R.

Mendelian randomisation

Mendelian randomisation (MR) is an approach that examines genetic variants in association with an exposure and outcome to determine causality. In this study if a significant genetic correlation ($P < 0.05$) was found (indicating pleiotropy) it was carried forward into a two-sample MR analysis. We utilised the 'MendelianRandomization' package (v0.2.0) in R to conduct both an Inverse-Variance Weighted (IVW) analysis and MR-Egger regression.[21] In brief, the IVW method incorporates multiple SNPs as a vector of instrumental variables (IVs) and carries out weighted linear regression analysis between the IVs vector—outcome and IVs vector—exposure. The analysis is weighted on the inverse variance of the IVs vector—outcome association and the intercept constrained to zero. We utilised the effect beta from the genome-wide significant variants from the original ENIGMA GWAS as the association between variants and exposure and the effect beta from the same variants in the PGC GWAS as the association between the variant and outcome. We also tested the association between the variants and MDD in the GS:SFHS, UK Biobank and ELSA (Supplementary Materials). If the variant was not available in a data set, that data set was either removed or the variant in highest LD available in both data sets was used. The constraint of the intercept at zero in IVW, however, assumes that all IVs are valid. As this is not always the case, if a significant association ($P < 0.05$) was indicated in IVW analysis, sensitivity analysis with MR-Egger regression was conducted. As MR-Egger regression does not constrain the intercept, it is therefore is not biased by invalid IVs.[38] The same effect beta's and standard errors were utilised in the MR-Egger regression. For further details on the methodology see Supplementary Materials.

BUHMBOX

To further explore correlation between subcortical volume and MDD, we utilised the technique BUHMBOX.[22] This technique tests for the presence of true pleiotropy and genetic subgroup heterogeneity within cases of a disease phenotype (phenotype A) by measuring pairwise correlations of risk alleles with another trait (phenotype B). The presence of phenotype B risk alleles across all phenotype A cases and not phenotype A controls provides evidence of true pleiotropy, whereas subgroup heterogeneity is implicated if phenotype B risk alleles are enriched in a subgroup of phenotype A cases. Pairwise correlations are combined to generate a BUHMBOX test statistic for clinical heterogeneity. In this study, if a significant genetic correlation ($P < 0.05$) was found, we utilised BUHMBOX to further dissect the genetic relationship between regional brain volume and MDD. We examined risk alleles associated with the ENIGMA regional brain volumes as phenotype B with MDD phenotypes in GS:SFHS, UK Biobank and ELSA as phenotype A. In order to minimise bias caused by related individuals in GS:SFHS, an unrelated subsample was used comprising 5659 individuals (786 MDD cases). LD pruning was conducted using PLINK 1.90[39] using --indep-pairwise with $r^2 > 0.1$ and a window size of 50 SNPs and a sliding winding of five SNPs. The first 4 PCs were fitted in GS:SFHS and ELSA and the first 15 PCs were fitted in UK Biobank to account for additional heterogeneity.

Power Analyses

Power analyses for the genetic correlations (r_G), calculated using LD score regression, were completed using the GCTA-GREML power calculator.[40] As LD score regression utilises summary statistics and GCTA, the individual genotype data, true power is likely to be slightly lower; however, the GCTA-GREML power calculator gives a close estimate. Results of the power analysis are presented in Supplementary Table S1.

Simulations of genetic correlations varying from 0.1 to 0.5 between the brain volume and MDD indicated that, at $r_G = 0.5$, we had power to detect an association ($P < 0.05$) in all regions. However, genetic correlation was found to be much lower for many of the regions and therefore we only had adequate power to detect a correlation between hippocampal volume and MDD (power = 93%). For the remaining regions, a power curve was conducted to demonstrate the size of the sample needed for sufficient power (Figure 1 and Supplementary Figure S2). Results indicate that an additional ~15 000 sample increase in both ENIGMA and PGC samples would be needed to detect significant genetic correlations between MDD and either putamen or ICV at the estimates reported in this analysis. To demonstrate significant genetic correlation between MDD and either nucleus accumbens or pallidum volumes would require sample size increases of greater than 100 000 in both samples. Power curves of simulated genetic correlations (varying from 0.1 to 0.5) were also constructed to identify the genetic correlation that we would have had power to detect at the current sample size. Results indicate that this sample had adequate power to detect a genetic correlation of at least 0.24 for putamen, 0.26 for nucleus caudate, 0.33 for both pallidum and ICV, 0.37 for hippocampus, 0.38 for thalamus and 0.49 for nucleus accumbens.

Power analysis of PRS were completed using AVENGEME.[41] Markers were assumed to be independent and 5% of SNPs were assumed to have an effect in the training sample. Genetic covariance values were taken from the LD score regression analysis, however, no value could be computed for amygdala therefore three hypothetical covariances were tested; 0.50, 0.25 and 0.10. Results of the power analysis are presented in Supplementary Table S3.

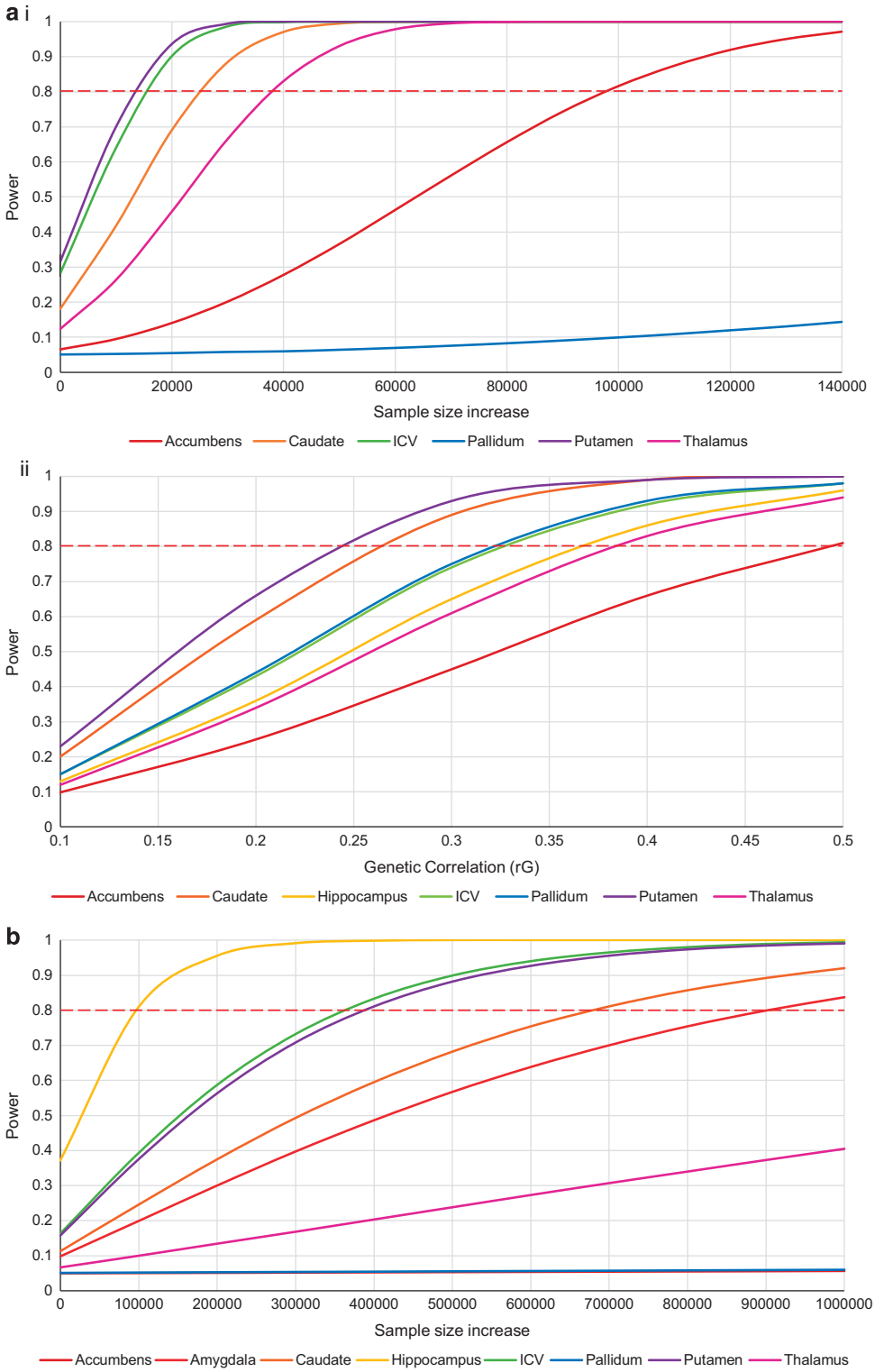

Figure 1. Power curves were calculated with starting point 0 as the sample size in our analysis. For the genetic correlation power analysis the power curves demonstrate (**a**(i)) the sample size increase needed for detecting a significant association at the estimates reported in this analysis when both samples were increased equally and, for MDD, the proportion of cases and controls was kept constant and (**a**(ii)) the genetic correlation that there would be power to detect at the sample size reported in this analysis. For PRS power analysis (**b**) the sample size for the training set (ENIGMA) was kept constant while the target set sample size was increased. Amygdala was assumed to have a $r_G = 0.25$ for the PRS power analysis. Hippocampus had adequate power in the genetic correlation analysis and therefore was not included in the power curve. ICV, intracranial volume; MDD, major depressive disorder; PRS, polygenic risk scoring.

Table 1. SNP-based heritability (h2) and genetic correlation (r_G) of subcortical brain regions and ICV with MDD

	SNP heritability			*Genetic correlation with MDD*			
Brain region	*SNP* h^2	*s.e.*	*Z-score*	r_G	*s.e.*	*Z-score*	P
Nucleus accumbens	0.0855	0.0438	1.95	0.0458	0.210	0.218	0.828
Caudate nucleus	0.253	0.0432	5.86	0.0752	0.130	0.580	0.562
Hippocampus	0.137	0.0481	2.85	0.460	0.200	2.30	0.0213
ICV	0.167	0.0462	3.61	0.123	0.166	0.739	0.460
Pallidum	0.171	0.049	3.49	−0.0077	0.158	−0.0491	0.961
Putamen	0.297	0.051	5.82	0.0986	0.118	0.834	0.404
Thalamus	0.125	0.0401	3.12	−0.0808	0.177	−0.457	0.648

Abbreviations: ICV, intracranial volume; MDD, major depressive disorder; SNP, single-nucleotide polymorphism. The heritability of amygdala was nonsignificant and therefore removed from subsequent analysis. The *P*-values shown are uncorrected for multiple testing (for the results corrected for multiple testing see Supplementary Table S2).

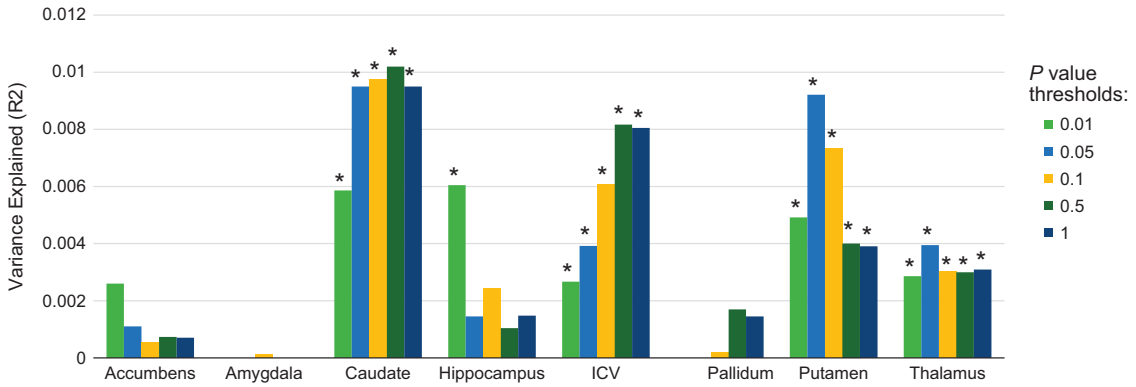

Figure 2. Significant *P*-values (< 0.05) are indicated with asterisk (*). Nucleus accumbens, amygdala and pallidum PRS were not significantly associated with their respective volume at any threshold. ICV, intracranial volume; PRS, polygenic risk scoring.

Despite a study size of 49 576 individuals, PRS was under-powered in all analyses. Highest power in the meta-analyses was for hippocampal volumes (37%). Low SNP heritability and low covariance between traits account for the low power in the meta-analyses. In the PRS analysis on their own trait, highest powered was the putamen (23%) at a *P*-value threshold of 1. In this analysis a small sample size of 968 individuals likely reduced power. We therefore conducted a power curve for both the meta-analysis and PRS in their own trait to indicate the sample size that would be necessary to have adequate power (Figure 1 and Supplementary Figure S3). Power curves indicate that a sample increase of ~100 000 individuals in the target set would be sufficient power for hippocampus PRS associated with MDD, however nearly 900 000 for amygdala assuming a covariance of 0.25 and an increase of over 1 million participants for nucleus accumbens, pallidum and thalamus.

RESULTS

Genetic correlation

Using LD score regression, we calculated SNP-based heritability estimates for the seven subcortical regions and ICV with MDD, utilising summary data from GWAS completed by ENIGMA[17] and PGC,[33] respectively. The estimate of the SNP heritability for the amygdala was non-significant and therefore the amygdala was not included in any further analysis. SNP heritability estimates for the remaining subcortical volumes ranged from the SNP $h^2 = 0.0855$ (s.e. = 0.0438) for the nucleus accumbens to SNP $h^2 = 0.297$ (s.e. = 0.051) for the putamen (Table 1). MDD SNP heritability was calculated at 0.204 (s.e. = 0.0386). Genetic correlation between each subcortical region and ICV with MDD was then calculated. Hippocampal volume demonstrated significant genetic correlation with MDD ($r_G = 0.460$, s.e. = 0.200, $P = 0.0213$; Table 1), although this did not survive multiple testing correction using false discovery rate adjustment (Supplementary Table S3). No other subcortical volume or ICV was genetically correlated with MDD.

Polygenic risk score

Association between regional brain volume PRS and its respective volume. Subcortical and ICV PRS were calculated in UK Biobank to examine the association between each regional volume PRS and its own volume. PRS were positively associated with their respective volume in four of the eight structures across the five *P*-value thresholds; caudate nucleus, ICV, putamen and thalamus. In addition, hippocampus was significantly associated at a *P*-value threshold of 0.01 only. These results retained significance after multiple test correction across the five thresholds, however only raw *P*-values have been reported. Nucleus accumbens, amygdala and pallidum PRS did not demonstrate any association with their respective volume. The variance explained by PRS was small for all volumes, with the largest reported in the caudate nucleus ($R^2 = 0.0102$, $\beta = 0.117$, $P = 1.08 \times 10^{-4}$; Figure 2 and Supplementary Table S2).

Association between regional brain volume PRS and MDD. Structural PRS were selected at the threshold that best predicted its own volume (nucleus accumbens = 0.01, amygdala = 0.1, caudate nucleus = 0.5, hippocampus = 0.01, ICV = 0.5, pallidum = 0.5, putamen = 0.1, thalamus = 0.05) and tested for prediction of MDD status. No PRS for any volume was significantly associated with MDD status in any of the cohorts (Supplementary Table S4). In

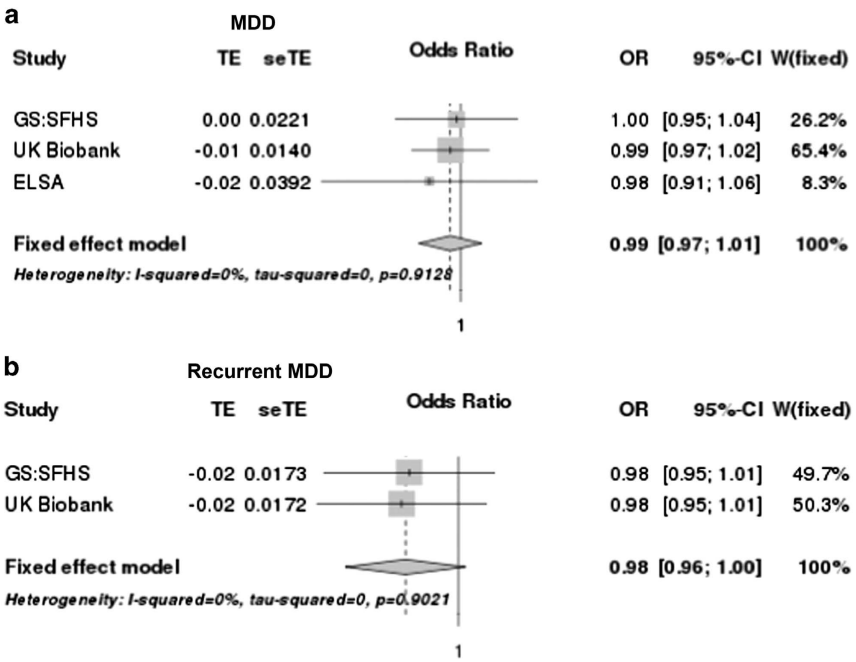

Figure 3. Both plots demonstrate a negative correlation with MDD and recurrent MDD with no heterogeneity between cohorts but neither plot reaches statistical significance. CI, confidence intervals; GS:SFHS, Generation Scotland: Scottish Family Health Study; MDD, major depressive disorder; OR, odds ratio; seTE, standard errors; TE, treatment effect (regression beta's); W(fixed), weight of individual studies in fixed effect meta-analysis.

order to increase power, we completed a meta-analysis of the summary association statistics from three cohorts. No evidence of heterogeneity was identified in any of the meta-analyses. We found no association between any structural PRS and MDD (Figure 3a and Supplementary Figure S4).

Association between hippocampus volume PRS and MDD traits. Association between hippocampal volume and recurrent MDD and early illness onset has been previously reported.[5,36] We therefore examined MDD phenotypes in association with hippocampal volume PRS in GS:SFHS and UK Biobank, these data were not available for the ELSA cohort. There was no association between hippocampal PRS and recurrent MDD (OR = 0.98, $P = 0.0850$) (Figure 3b). Further, hippocampal volume PRS was not significantly associated with number of episodes ($\beta = -0.00390$, $P = 0.425$), MDD duration ($\beta = -0.00110$, $P = 0.414$) or age of onset ($\beta = 0.0142$, $P = 0.291$; Supplementary Figure S5).

Mendelian randomisation

To further examine the nominally significant genetic correlation between hippocampus and MDD, MR analysis was performed to test for a directional association between hippocampal volume and MDD. Only two genome-wide significant variants were identified in the original ENIGMA GWAS (rs61921502 and rs77956314) and these SNPs were only present in two of the cohorts (GS:SFHS and UK Biobank). To obtain a value for variant—outcome in the PGC data set, we selected the SNPs in highest LD with the two causal variants that were available in both ENGIMA and PGC summary statistics; rs17765551 and rs7294919. We also conducted the analysis using values obtained with the causal variants from meta-analysis with GS:SFHS and UK Biobank. IVW analysis did not identify evidence for a causal association between hippocampus variants and MDD in using either PGC MDD as the outcome nor GS:SFHS and UK Biobank MDD (Supplementary Table S5), it was therefore not necessary to carry this forward into the MR-Egger regression model.

BUHMBOX

To further investigate whether the genetic correlation found between hippocampus volume and MDD could be due to genetic subgroup heterogeneity, we utilised the software package BUHMBOX. SNP subsets were used that were associated with hippocampal volume at a threshold of $P < 1.0 \times 10^{-3}$ and had to be present in all individuals per cohort therefore 388, 504 and 386 SNPs, and 5659, 7017 and 4118 individuals remained in GS:SFHS, UK Biobank and ELSA, respectively. Clinical heterogeneity was found in GS:SFHS MDD cases (Z-score = 2.78, $P = 0.00281$), demonstrating excessive pairwise correlations between risk alleles for hippocampus volume and a subgroup of MDD cases. This survived false discovery rate multiple testing correction, however, this finding was not replicated in either UK Biobank or ELSA (Table 2).

DISCUSSION

Previous studies have reported phenotypic associations between brain volumes and MDD. In this study, we investigated whether there was evidence of shared genetic architecture between subcortical brain volumes and ICV with MDD. Results from the genetic correlation analysis indicate that hippocampal volume and MDD are partially influenced by common genetic variants ($r_G = 0.46$, s.e. = 0.200, $P = 0.0213$), although this did not survive correction for multiple testing. This positive genetic correlation is novel, so far as any of the authors are aware, however Mathias *et al.*[42] demonstrated significant negative correlation between recurrent MDD and right hippocampal volume measured via linkage analysis in a sample of 893 individuals. No other brain regions' volume showed evidence of shared genetically aetiology with MDD. Our sample size was adequate to detect a correlation at $r_G = 0.5$, however, at the values reported in this study, we were underpowered in all other brain volumes (excluding hippocampus). Power analyses indicate that we had insufficient power to detect weak to modest genetic correlations in this study. Although we were able to demonstrate lack of strong genetic correlations between regional brain volumes (excepting hippocampus) and

Table 2. BUHMBOX results for hippocampus volume and MDD in GS:SFHS, ELSA and UK Biobank

	BUHMBOX P	$P_{corrected}$	*Z-score*	N	*Cases*	*Controls*	*SNPs* N	*Pleiotropy* P	*Pleiotropy* β	*Pleiotropy s.e.*
GS:SFHS	**0.00281**	**0.00843**	**2.77**	**5659**	**786**	**4873**	**388**	0.0890	−0.0448	0.0263
UK Biobank	0.893	0.893	−1.24	7017	2316	4701	504	0.926	−0.00128	0.0138
ELSA	0.678	0.893	−0.462	4116	544	3572	386	0.650	0.0144	0.0316

Abbreviations: GS:SFHS, Generation Scotland: Scottish Family Health Study; ELSA, English Longitudinal Study of Ageing. Significant results ($P < 0.05$) are shown in bold.

MDD, we cannot with confidence exclude the possibility that they are weakly to modestly correlated. Therefore, further analysis utilising larger sample sizes (a minimum of 15 000 increase in both samples) would be necessary to draw confirmatory conclusions.

Brain volume PRS were not associated with their own volume in three out of the eight structures and was only associated with hippocampal volume at *P*-value threshold 0.01. This is likely due to the analysis being underpowered to detect an association in a sample size of 968 participants. Of the PRS that were associated with their phenotype, the largest proportion of variance explained was 1% with the majority predicting ~0.6%. The proportion of variance explained is therefore very low although this is fairly common in PRS studies[43] with one of the largest explained variance by PRS reported in schizophrenia (~7% on the liability scale).[44]

Meta-analysis of data from three studies, totalling 49 576 individuals including 11 552 cases, found no evidence of association between any regional brain volume PRS and MDD, including the hippocampus. As previous neuroimaging evidence suggests that decreased hippocampal volumes could occur as a cause or consequence of recurrent depressive episodes and early illness onset,[5,36] we examined hippocampal volume PRS associations with recurrent MDD, number of episodes, MDD duration and age of onset but we observed no significant associations. Despite the PRS meta-analysis being the largest analysis to date examining genetic scores for brain volume and MDD, it was severely underpowered; therefore, we can draw no confirmatory conclusions about the genetic overlap between any structure and MDD from this analysis. The apparent discrepancy between PRS and our finding LD score regression is likely due to this lack of power, however, as LD information is utilised in LD score regression and SNPs are pruned in PRS calculation, it is also possible that the 'loss of information' involved in calculating PRS contributed. Previous simulation studies have demonstrated that predictive capabilities of PRS are greatly enhanced when utilising LD information.[45] This implies that LD pruning may be removing causal SNPs and those more closely tagging causal variants, resulting in a loss of information and predictive accuracy.

To further dissect the positive genetic correlation between hippocampus and MDD, we utilised MR and BUHMBOX techniques. MR was used to determine the causality of genetic variants in association with hippocampal volume and MDD. We did not detect a causal association, however, there were only two genome-wide significant SNPs associated with hippocampal volume in the original GWAS. Larger numbers of associated variants increase power in MR analysis[38] therefore this was likely a contributing factor in this analysis. We also applied BUHMBOX to investigate genetic subgroup heterogeneity and detected evidence of a subgroup in MDD cases within GS:SFHS. We did not replicate this finding in UK Biobank or ELSA, however, MDD cases are not defined using a clinical measure in these cohorts, whereas GS:SFHS cases are defined using DSM-IV criteria. The PGC MDD definition also most closely matches that of GS:SFHS MDD, although the GS:SFHS sample was population-based rather than identified from a clinically ascertained samples. This could explain why the findings were associated with these cohorts and not the others. The observed lack of replication may then be due to factors related to ascertainment differences and should therefore be replicated in a clinically determined MDD sample.

We conclude that hippocampal volume and MDD may share common genetic factors, although this result did not withstand multiple testing correction. Animal models have previously demonstrated that increased stress can drive decreased hippocampal neurogenesis (and therefore increased atrophy)[46] and this reduced neurogenesis can lead to depressive-like symptoms.[47] Stress is a well-established environmental risk factor associated with MDD[48] and the inhibition of glucocorticoid receptors has been shown to normalise hippocampal neurogenesis[49] and relieve symptoms in psychotic major depression.[50] Furthermore, increased duration of depression has also been related to more pronounced hippocampal reductions.[51] Our results however indicated a positive genetic correlation suggesting that genetic variants determining larger hippocampal volume may be risk factors for MDD. The clinical heterogeneity found utilising BUHMBOX in GS:SFHS could provide a possible explanation for the deviation from literature. If genes for larger hippocampal volume are present in a subgroup of MDD only, then it is possible that hippocampal volume atrophy could be associated with a different subgroup of individuals that are affected through more environmental pathways. Hippocampal volume has been demonstrated to be more highly impacted by the environment than other brain regions[52] and is associated with many environmental factors, for example, stress,[48] increased exercise training[53] and jet lag.[54] It is therefore possible that the previously reported decreased hippocampal volume associated with MDD is due to multiple episodes of depression and that this positive genetic correlation is due to a role in MDD susceptibility earlier in brain development. In fact, it has been previously demonstrated that first episode MDD subjects exhibited marginally larger hippocampal volumes in comparison to healthy controls.[55] This could also provide explanation for the opposing negative genetic correlation finding by Mathias *et al.*[42] as they examined recurrent MDD. However, it should be noted that this study did not demonstrate significant hippocampal atrophy in analysis including controls,[55] which has been similarly shown in another study.[56] Given that this positive correlation could be associated with a subgroup of MDD cases, it is also possible that this is hindering investigations into hippocampus and all MDD cases. Hippocampal volume changes are also widely associated with other psychiatric disorders such as schizophrenia. A similar analysis that examined the genetic correlation between subcortical volumes and schizophrenia found no significant correlations.[57] This is suggestive that the genetic correlation observed could be specific to hippocampal volume in MDD. However, these results are only indicative of a genetic correlation between the two traits and further research would be necessary to provide confirmative evidence.

A number of limitations of this study should be noted; first, this study only explored the effects of common genetic variants and it

may be important to examine rarer variants to generate a more complete picture, although this will require larger sample sizes. Second, the lower heritability, higher prevalence and likely heterogeneity of MDD results in less precise estimates of marker weights from GWAS,[58] decreasing the power to detect genetic correlations with other phenotypes. Power of the PRS is limited also by the size of the initial ENIGMA GWAS ($n = 11\,840$), larger discovery sample sizes greatly improve the accuracy of PRS.[44,59] Therefore, larger genome-wide analysis would be necessary to generate confirmatory conclusions. Third, the estimates for SNP heritability, calculated using LD score regression, were lower than have been previously described.[60] LD score regression has been utilised previously to calculate SNP heritability of subcortical volumes using the ENIGMA summary data with similar low estimates reported.[57]

Despite these limitations, we provide some evidence of a positive genetic correlation between hippocampal volume and MDD and an indication of MDD subgroup heterogeneity, however, the genetic correlation did not survive multiple testing correction and the subgroup heterogeneity was not replicated. We did not demonstrate an association utilising PRS techniques, however, low power, low explanation of variance and loss of LD information were notable limitations. Although we demonstrate a potential genetic relationship between hippocampal volume and MDD in a subgroup of individuals, we believe one of the most important outcomes for the current study is in the planning for future studies. Sample sizes of ~150 000 individuals will be needed to have sufficient statistical power (>0.8) to detect shared genetic architecture between MDD and hippocampal volume using PRS, using data sets similar to the one studied. The other regional brain volumes ranged from needing an additional sample size of ~400 000 to in excess of 1 million individuals. Alternatively, further studies may utilise data from further releases of the ENIGMA consortium, including larger numbers of participants and more accurately determined SNP effect sizes. Further research into subgrouping in the association between hippocampus and MDD may also be beneficial.

CONFLICT OF INTEREST

AMM has received financial support from Pfizer (formerly Wyeth), Janssen and Lilly. IJD and DJP were participants in UK Biobank. The remaining authors declare no conflict of interest.

ACKNOWLEDGMENTS

This investigation was supported by the Wellcome Trust 104036/Z/14/Z (STRADL, Stratifying Resilience and Depression Longitudinally). Generation Scotland received core funding from the Chief Scientist Office of the Scottish Government Health Directorate CZD/16/6 and the Scottish Funding Council HR03006. We thank all families, practitioners and the Scottish School of Primary Care involved in the recruitment process as well as the entirety of Generation Scotland team; interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, healthcare assistants and nurses. We are grateful towards the Dr Mortimer and Theresa Sackler foundation for the financial support for this work. This research has been conducted using the UK Biobank resource and we would therefore like to thank all participants and coordinators in this cohort. The UK Biobank study was conducted under generic approval from the NHS National Research Ethics Service (approval letter dated 17 June 2011. Ref 11/NW/0362). Samples from the English Longitudinal Study of Ageing DNA Repository (EDNAR), which receives support from the National Institute on Aging (NIA) and the Economic and Social Research Council (ESRC), were used in this study. We thank contributors and the ELSA participants. IJD is supported by MRC and BBSRC funding to the University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology (MR/K026992/1).

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)

3.3. Chapter Conclusion

This study largely indicates a lack of genetic correlation between subcortical volumes and MDD, excluding hippocampus which would need to be additionally examined in an independent dataset before any confirmatory conclusions can be drawn. It is possible that this nominal association in hippocampal volume is a genetic subgroup (as indicated by BUHMBOX in GS:SFHS), however given this results contradicts previous direction of effect it is also very possible that this correlation is type I error. Nevertheless, lack of a genetic correlation for hippocampal volume does not disclude the possibility of environmental impact. In fact, a 2009 meta-analysis found that hippocampal volume reductions occur after MDD onset (McKinnon, Yucel, Nazarov, & MacQueen, 2009) and childhood trauma has been associated with small hippocampal volume in MDD (Vythilingam et al., 2002). This study counters the previous reports that show an association between candidate genes for MDD and brain volumes. The lack of associations provides several different possible interpretations: (1) brain volumes and MDD are weakly to modestly correlated at a level that we did not have the power to detect with the current sample, (2) brain volume genetic factors are not associated with risk for MDD but non-genetic factors are, or (3) regional brain volumes are not associated at all with MDD pathology. Further examination of environmental effects in regional brain volume and MDD will elucidate more about their association.

Subcortical brain volumes have also been associated with antidepressant response therefore a similar analysis exploring antidepressant resistance and subcortical volumes would be informative. Nevertheless, given that this analysis was underpowered (in all but hippocampus), significantly larger samples for antidepressant response would be needed as the largest current GWAS is only ~2,900 individuals (Tansey et al., 2012).

Future studies should investigate the marginal evidence for MDD subgrouping as this may explain the inconsistent findings in MDD and neuroimaging studies. Nonetheless, this study generally supports a lack of genetic association between regional brain volume and MDD.

Chapter 4. The Genetics of Antidepressant Resistance.

4.1. Background

In Chapter 1, the high heterogeneity, comorbidities and possible biological mechanisms that underlie MDD were explored. Given that no individual biological theory has adequately explained MDD, it is possible that the diagnosis may group individuals with diverse aetiologies. Furthermore, many of the theories on MDD pathology overlap with findings in antidepressant non-response e.g. higher inflammatory markers indicate a higher likelihood for non-response (Uher et al., 2014). Therefore, it is also possible that the causes of antidepressant non-response may define different aetiological MDD subgroups. Examining pathways to non-responsiveness could therefore aid in stratification and explication of biological mechanisms.

Identifying genetic variants associated with antidepressant non-response may facilitate our understanding of antidepressant mechanisms, stratify individuals into more aetiologically circumscribed subgroups and inform the construction of personalised treatments. The most common methodology for studying non-response is a continuous assessment of reduction in baseline depression symptoms during treatment measured by scales such as the Montgomery-Asberg Depression Rating (MADRS) and Hamilton Rating Depression Scale (HRSD). Nevertheless, these studies are conducted as clinical trials which are highly regulated, require significant resources and staffing and are prone to under-recruitment and premature termination. Clinical trials are therefore prone to small sample sizes (Moaddeb & Haga, 2013; Naudet et al., 2011) and are likely to be underpowered, especially for genetic studies. Alternate approaches to obtaining sufficient sample sizes for genetic studies of treatment response are therefore needed. Both self-report response data and electronic health records provide possible alternatives as these represent cheaper substitutes that can be applied with relative ease to large samples. However, these techniques typically provide less detailed information about individual drug response than a continuous approach. Previous studies utilising these approaches have deduced binary responder/non-responder or responder/resistant status. For example, 23andMe applied information from a self-

report questionnaire on response whereby assessment of an individual's response to an antidepressant was ranked on a scale from "Not at all" or 0 to "A fair amount" or 3. Treatment non-response was then defined as a score less than or equal to 1 and was used to deduce non-response to selective serotonin reuptake inhibitors (SSRIs) and bupropion and additionally treatment resistance (which was non-response to more than 2 antidepressants and additionally never reporting a score of more than 3) (Q. S. Li et al., 2016). Additionally, using electronic health records, treatment resistant depression was defined in an antidepressant-user population as non-response to two or more antidepressants using a previously validated natural language processing (NLP) tool (O'Dushlaine et al., 2014). The NLP tool utilised longitudinal data from clinic visits and prescription records to define treatment resistance and then compared their outcomes with the International Classification of Diseases 9 (ICD-9) codes reporting an area under the curve (AUC) of 0.85-0.88 (Perlis et al., 2012).

The largest current genome-wide association study (GWAS) on antidepressant response is a meta-analysis conducted by NEWMEDS and STAR*D that measured response continuously as reduction in symptoms using both the MADRS and HRSD-17 scales (Tansey et al., 2012). No significant genetic associations or pathway enrichment was detected and an additional PRS analysis found no significant prediction of NEWMEDS PRS in STAR*D. The largest current GWAS on antidepressant treatment resistance (measured as a binary outcome) was completed by 23andMe using self-report data (detailed above) but similarly found no significantly associated genetic variants (Q. S. Li et al., 2016). Nonetheless, analysis refined to specific antidepressant classes have described associated variants; a meta-analysis of over 1,354 individuals identified a variant located in an intergenic region on chromosome 5 (near pseudogene *LOC643401*) associated with 2-week outcomes with citalopram and escitalopram (GENDEP Investigators et al., 2013). Additionally, a further 23andMe analysis found an intergenic variant located between genes *GPRIN3* and *SNCA* associated with bupropion response (Q. S. Li et al., 2016); bupropion is a noradrenaline-dopamine reuptake inhibitor but is not currently indicated for MDD in the National Health Service (NHS) (Joint Formulary Committee, 2017). Furthermore, through gene-set enrichment, they identified circadian rhythm, long-term depression and vascular endothelial growth factor pathways.

This chapter will explore treatment resistance (TR) in a population-based cohort using prescription records gained by linkage to NHS electronic health records whilst also examining antidepressant resistance in a semi-quantitative scale, stages of resistance (SR). This analysis will be, in part, a proof of principle study to demonstrate the use of health records in large biobanks, which have becoming increasing popular in recent years. Genome-wide association study, gene and gene-set enrichment were conducted. Moreover, to explore whether any traits that have previously been associated with depression could meet some of the criteria for intermediate phenotypes for treatment resistance, polygenic risk score (PRS) analysis and genetic correlations were also performed. The content for this chapter has been summarised in a manuscript entitled "Genome-wide association study of antidepressant treatment resistance in a population-based cohort using health service prescription data and meta-analysis with GENDEP" which has been submitted for publication. I confirm, as the first author, that I carried out the analysis of the data (excepting the GENDEP GWAS which was conducted by GENDEP themselves due to access restrictions) and wrote the following paper.

4.2. Genome-wide Association Study of Antidepressant Treatment Resistance in a Population-based Cohort using Health Service Prescription Data and Meta-analysis with GENDEP

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Number of words (main text)	3,169
Number of references	57
Number of tables	3
Number of figures	2

4.2.1. Abstract

Antidepressants demonstrate modest response rates in the treatment of major depressive disorder (MDD). Despite previous genome-wide association studies (GWAS), the genetic factors underlying non-response are unknown. Using prescription data in a population and family-based cohort (Generation Scotland: Scottish Family Health Study; GS:SFHS), we sought to define a measure of (a) treatment resistance and (b) stages of resistance by inferring antidepressant switching as non-response to treatment. GWAS were conducted separately for treatment resistance in GS:SFHS and the Genome-based Therapeutic Drugs for Depression (GENDEP) study and then meta-analysed (meta-analysis n=4,213, cases=358). For SR, a GWAS on GS:SFHS only was performed (n=3,452). Additionally, we conducted gene-set enrichment, polygenic risk scoring (PRS) and genetic correlation analysis. We did not identify any significant loci, genes or gene-sets associated with treatment resistance or stages of resistance. Significant positive genetic correlations of treatment resistance and stages of resistance with neuroticism, psychological distress, schizotypy and mood disorder traits were identified. These findings suggest that larger sample sizes are needed to identify the genetic architecture of antidepressant treatment response, and that population based observational studies may provide a tractable approach to achieving the necessary statistical power.

4.2.2. Introduction

Major depressive disorder (MDD) is a disabling condition with a high global impact (Collaborators, 2015; Ustün et al., 2004). Antidepressants are the first-line treatment for MDD patients but response is modest with only approximately 50% achieving remission after completing two treatments (A. Rush et al., 2006). The mechanisms underlying antidepressant resistance remain elusive, but are of key value if more effective therapies are to be identified and developed.

Genome-wide association studies (GWAS) of antidepressant treatment response have yet to establish any replicated genetic variants (Biernacka et al., 2015; Cocchi et al., 2016; Garriock et al., 2010; Ising et al., 2009; Myung et al., 2015; Tansey et al., 2012; Uher et al., 2010). Two large meta-analyses similarly reported no genome-wide significant associated variants. The first, a meta-analysis of the GENDEP (Genome-based Therapeutic Drugs for Depression), MARS (Munich Antidepressant Response Study) and the STAR*D (Sequenced Treatment Alternatives to Relieve Depression) (GENDEP Investigators et al., 2013) studies comprised of 2,256 individuals, and the second, between the NEWMEDS (Novel Methods Leading to New Medications in Depression and Schizophrenia) and STAR*D (Tansey et al., 2012) projects comprised of 2,897 MDD individuals. An additional analysis in the first meta-analysis restricted to citalopram or escitalopram did, however, identify an intergenic variant (5q.15.1) (GENDEP Investigators et al., 2013). The largest GWAS to date examining treatment resistance (TR=1,311) versus responders (n=7,795) was conducted by 23andMe utilising self-report information, however, no significantly associated genetic variants were reported. Nevertheless, one variant (4.q22.1) was found in association with bupropion response (Q. S. Li et al., 2016). Numerous candidate genes have also been investigated but the results are inconsistent (Fabbri et al., 2014). Furthermore, the largest polygenic risk score (PRS) analysis in antidepressant response to date, (which utilised GENDEP/STAR*D data) yielded no significant associations for response itself, MDD or schizophrenia (García-González et al., 2017).

Discovering genomic variants associated with resistance to antidepressants could advance personal treatment, help identify resistant individuals earlier and inform our understanding of MDD. A recent systematic review reported non-response was

associated with illness severity including higher suicide risk, number of hospitalisations and antidepressant dosage, but not cognitive ability (De Carlo, Calati, & Serretti, 2016). In fact, several phenotypic associations have been found in treatment resistant individuals; more comorbidities and suicide attempts (Amital, Fostick, Silberman, Beckman, & Spivak, 2008), increased neuroticism and decreased extraversion, openness and conscientiousness (McGirr, Van den Eynde, Chachamovich, Fleck, & Berlim, 2014; Takahashi et al., 2013). Identifying genetic loci may therefore help to identify resistant individuals earlier and enable timelier intervention.

Pharmacogenetic studies are particularly susceptible to small sample sizes (Bacanu, Whittaker, & Nelson, 2012) and low statistical power. Numerous studies have indicated the need for large sample sizes in genetic studies (Baker, 2014; Bergen & Petryshen, 2012). The recent 23andMe study maximised sample size by utilising self-report questionnaires (Q. S. Li et al., 2016), whilst other groups have examined treatment resistance in both MDD or schizophrenia by using prescription data (O'Dushlaine et al., 2014; Ruderfer et al., 2016; Wimberley et al., 2016).

In the present study, we employed a complementary approach utilising prescription data in a population and family-based cohort (Generation Scotland: Scottish Family Health Study; GS:SFHS) to define a dichotomous and a semi-quantitative measure of antidepressant resistance; treatment resistance and stages of resistance, respectively. We conducted a GWAS of treatment resistance with meta-analysis with the GENDEP cohort and SR in GS:SFHS only and calculated narrow-sense heritability estimates. Gene and gene-set enrichment analysis on both traits were also conducted and we further examined genetic correlations. We also utilised PRS techniques to examine pleiotropy between the genetic liability of MDD, schizophrenia and bipolar disorder in treatment resistance and stages of resistance.

4.2.3. Methods

Cohort Description

Generation Scotland: Scottish Family Health Study

GS:SFHS is a family and population-based cohort of over 24,000 individuals (mean age=47.6, s.d.=15.4) within Scotland. Participants were eligible if they were aged above 18 years and had a first-degree relative also willing to participate in the cohort. Recruitment has been described in detail elsewhere (B. Smith et al., 2006; B. H. Smith et al., 2013). Genotype data were available for 20,032 participants and data on mood, cognitive function and personality traits were obtained through interview (see Supplementary Materials).

Prescription data were available through data linkage to the Prescribing Information System (PIS) administered by National Health Service (NHS) Scotland Information Services Division. Written informed consent for linkage was obtained for 98% of GS:SFHS. Further information regarding the prescription records are found in the Supplementary Materials (see **Supplemental Table S1** and **S2**). Records were excluded if the daily dose was below the minimum recommendations given by the British National Formulary (BNF) for MDD (Joint Formulary Committee, 2017) and the duration was below 6 weeks of continuous treatment (as this is considered adequate duration (Fava, 2003; Souery et al., 1999)). Following this pruning, we totalled the number of different antidepressants prescribed to each individual. This was then used as a measure of non-response, assuming that switching to a different antidepressant reflected failure or lack of clinical response. Drug switching due to side effects is expected to take place before the 6^{th} week of treatment. Individuals with schizophrenia, schizoaffective disorder and bipolar disorder were excluded (n=164).

Defining treatment resistance and stages of resistance

Treatment resistance was assessed in GS:SFHS using only individuals that had been prescribed at least one antidepressant at an adequate dose and duration (n=3,452). Case status for treatment resistance was defined as those individuals who had been prescribed more than 2 antidepressants providing 250 treatment resistant cases and 3,202 non-treatment resistant controls. There have been significant difficulties

defining treatment resistant depression in research but the general consensus is that it should be defined as non-response to more than two antidepressants (Conway, George, & Sackeim, 2016).

Individual response to antidepressants decreases with more unsuccessful trials (A. Rush et al., 2006), it has therefore been suggested that a semi-quantitative stages of resistance phenotype might be more informative than a dichotomous approach (Ruhé, van Rooijen, Spijker, Peeters, & Schene, 2012). Stages of resistance was defined as the number of different antidepressants prescribed given an adequate dose and duration. It was coded 1-4 with all individuals receiving more than 4 different antidepressants assigned a value of 4. This definition included 3,452 individuals on antidepressants (2557, 645, 186 and 64 on 1, 2, 3 and 4+ antidepressants, respectively).

Genome-based Therapeutic Drugs for Depression

GENDEP is a 12-week study that examined antidepressant response in 867 individuals (mean age=42.7, s.d.=11.6) taking escitalopram and noritriptyline. Response was measured by the Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979). Treatment resistance was defined as those who did not respond to more than 2 antidepressant therapies including GENDEP treatments and previous treatments (cases: 109, controls: 668), as described in a previous study (Iniesta, Malki, et al., 2016). A full description of the cohort is provided in **Supplemental Table S3**.

Genotyping, Imputation and Quality Control procedures

Generation Scotland: Scottish Family Health Study

Blood samples were stored and genotyped at the Wellcome Trust Clinical Research Facility, Edinburgh (www.wtcrf.ed.ac.uk). Details of the DNA extraction and genotyping have been given elsewhere (Kerr et al., 2013). Imputation to a combined reference panel of 1000 Genomes Phase 1 Version 3 and the UK10K haplotype reference panels was completed using Minimac3 and phasing was conducted utilising SHAPEIT2 (Nagy et al., 2017). Quality Control (QC) inclusion criteria were INFO > 0.9, missingness per single nucleotide polymorphism (SNP) or individual < 1%,

Hardy-Weinberg equilibrium (HWE) *P* value cut-off of $1x10^{-6}$, minor allele frequency (MAF) > 1%. 7,395,460 SNPs and 3,452 individuals (and 250 cases for treatment resistance) passed QC criteria.

Genome-based Therapeutic Drugs for Depression

DNA was extracted from blood samples and genotyped using the Illumina Human610quad bead chip (Illumina, Inc., San Diego). Imputation to the Haplotype Reference Consortium (HRC) data version 1 reference panel (S. McCarthy et al., 2016) was completed using Minimac3. QC exclusion criteria were poor imputation quality r2<0.3 (using the Markov Chain method (Y. Li, Willer, Ding, Scheet, & Abecasis, 2010)), missingness per SNP > 5%, missingness per individual > 3%, MAF < 1%, related individuals (identity-by-descent > 0.188). Individuals with gender discrepancies, abnormal heterozygosity and population outliers were excluded. 7,518,836 SNPs and 761 individuals (108 cases) passed QC criteria.

Statistical Analysis

Genome-wide association study

GWAS in GS:SFHS on treatment resistance and stages of resistance were completed utilising linear mixed model analysis in GCTA (Genome-wide Complex Trait Analysis) (J Yang et al., 2011). Age, sex and the first four multidimensional scaling (MDS) components were fitted as covariates and, to account for the family structure in GS:SFHS, genetic relationship matrices (GRMs) were fitted as random effects (see Supplementary Materials). To counter the loss of power that is caused by inclusion of a candidate SNP as both a random effect (in the GRM) and a fixed effect, the leave-one-chromosome-out method was utilised (J. Yang, Zaitlen, Goddard, Visscher, & Price, 2014). Due to the use of linear mixed models on a binary trait, treatment resistance, Taylor series transformation (Cortes et al., 2013) was used to convert beta and standard error values from the linear scale to the liability scale (see Supplementary Materials).

GWAS in GENDEP was completed on unrelated individuals utilising logistic regression in PLINK (Purcell et al., 2007). Models were corrected for age, centre,

baseline severity and the first four principal components, to control for population stratification.

Meta-analysis between GS:SFHS and GENDEP in treatment resistance was completed in METAL (Willer, Li, & Abecasis, 2010) with the inverse variance weighted method. A total of 7,120,598 SNPs were in common across both samples.

Gene and gene-set enrichment analysis

Gene and gene-set analysis were completed using MAGMA (v1.04) (de Leeuw, Mooij, Heskes, & Posthuma, 2015) (further details in Supplementary Materials). Individual level data were utilised for analysis of both treatment resistance and stages of resistance in GS:SFHS and summary statistics data used for analysis in GENDEP TR. Treatment resistance in GS:SFHS and GENDEP was then meta-analysed in MAGMA using fixed effect meta-analysis. To map SNPs to gene and biologically-meaningful gene sets, SNPs were annotated using NCBI 37.3 and, for the gene set analysis, gene-annotation files from BIOCARTA (http://www.biocarta.com/), KEGG (Okuda et al., 2008) and REACTOME (Croft et al., 2014; Fabregat et al., 2016) were taken from the Molecular Signatures Database (MSigDB) v5.2. Gene sets were corrected for multiple testing using the MAGMA default setting correcting for 10,000 permutations.

Pedigree-based heritability

Pedigree-based heritability of treatment resistance and stages of resistance was calculated in R using MCMCglmm (Hadfield, 2010). This was achieved by constructing a variance component matrix that takes into account all pedigree information and then fitting it into a univariate model as a random effect. MCMCglmm uses a Bayesian framework to estimate heritability. For TR, the logit link function was used to account for the binary nature of the phenotype.

Genetic correlation analysis

Genetic correlations were calculated using a bivariate analysis in ASReml-R (http://www.vsni.co.uk/software/asreml/). In order to assess the similarity between treatment resistance and stages of resistance, genetic correlation between the two was examined. Further, correlations between treatment resistance and stages of resistance

were examined with eight personality and cognitive variables; neuroticism, extraversion, schizotypal personality questionnaire (SPQ), mood disorder questionnaire (MDQ), general cognitive ability ('g', formed from a number of varied cognitive test scores), Scottish Index of Multiple Deprivation (SIMD), education and the general health questionnaire (GHQ). The ASReml-R method was utilised as it can account for the family structure in GS:SFHS. Genetic correlation measurements were calculated between pedigree-based heritabilities as the sample sizes were too small to conduct SNP-based correlations. More information on the variables and methods used can be found in the Supplementary Materials.

Polygenic risk scoring analysis

PRS were constructed utilising PLINK (Purcell et al., 2007). This method has been previously described (International Schizophrenia Consortium et al., 2009) and further information is available in the Supplementary Materials. Summary statistics taken from the Psychiatric Genomics Consortium (PGC) were used to construct PRS for MDD (unpublished data, see Supplementary Materials), schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) and bipolar disorder (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011) in the GS:SFHS cohort to examine genetic liability to the disorders in a treatment resistant population. PRS were reported across five *P* value thresholds (0.01, 0.05, 0.1, 0.5 and 1).

Association of PRS to the trait was analysed by linear mixed model analysis in ASReml-R (http://www.vsni.co.uk/software/asreml/) with treatment resistance or stages of resistance as the dependent variable and PRS as the independent variable. All models were adjusted for age, sex, and the first four MDS components and, to account for related individuals, an additive relationship matrix (expected relatedness derived from pedigree information) was fitted as a random effect. Wald's conditional F-test was used to derive *P* values for all fixed effects (see Supplementary Materials). Taylor series approximation (Cortes et al., 2013) was used for the treatment resistance variable, as above.

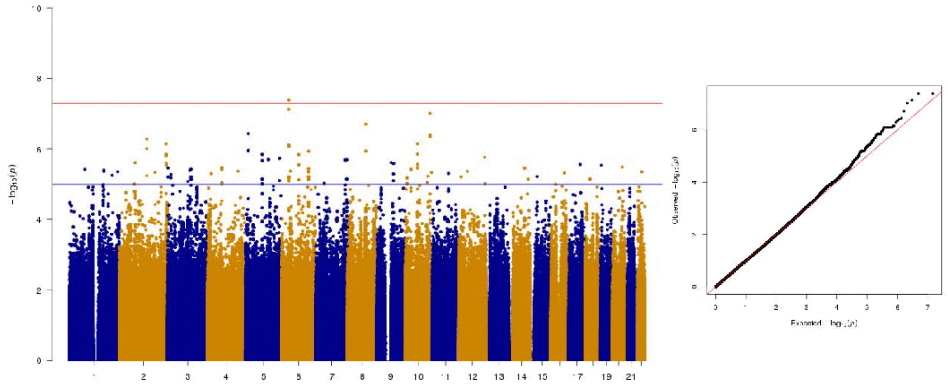
AVENGEME (Palla & Dudbridge, 2015) was used to calculate power in the PRS analysis assuming 5% of SNPs had an effect in the training sample and all markers were independent. Two theoretical covariances were tested at 0.5 and 0.25.

4.2.4. Results

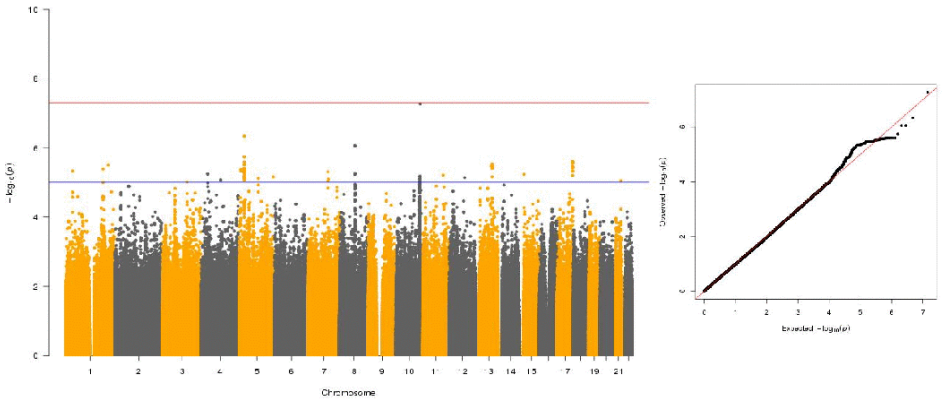
Genome-wide association study

In the treatment resistance meta-analysis of 4,213 individuals (cases=358, controls=3,855) no SNP reached genome-wide significance ($P > 5x10^{-8}$). The most significant SNP identified was an intergenic variant located at 10p26.13 (lead SNP rs188352979, $P=3.25x10^{-7}$, OR=2.87, CI=2.47-3.28; **Figure 4.1**).

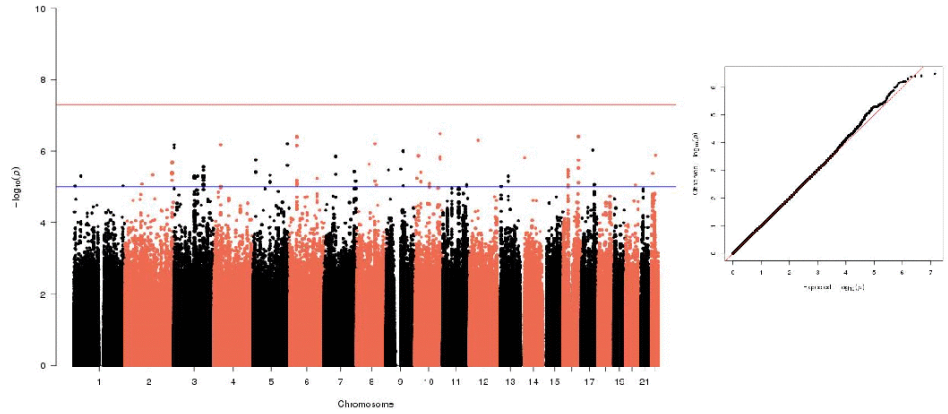
1. a)



b)

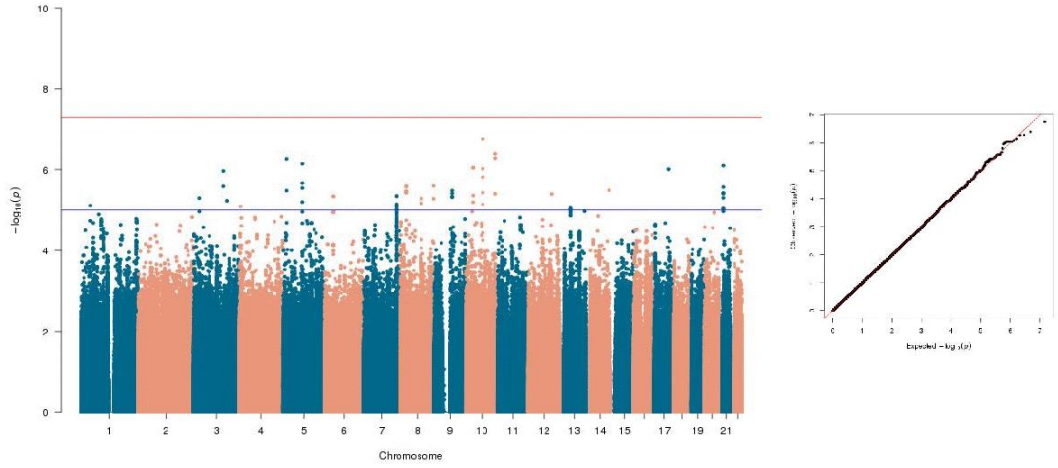


c)



4.1. Manhattan and Q-Q plots of the GWAS of antidepressant treatment resistance in (a) GS:SFHS, (b) GENDEP and (c) the meta-analysis between these two cohorts. Genome-wide significance level ($P<5x10^{-8}$) is represented by a red line and suggestive threshold ($P<1x10^{-5}$) is represented by a blue line.

In the GWAS of stages of resistance in GS:SFHS (n=3,452) no SNP reached genome-wide significance ($P > 5x10^{-8}$). The most significant SNP identified was an intergenic variant located at 10q22.1 (lead SNP rs116902282, $P=1.5x10^{-7}$, beta=0.49, s.e.=0.076; **Figure 4.2**).



4.2. Manhattan and Q-Q plots of the GWAS of antidepressant stages of resistance. Genome-wide significance level ($P<5x10^{-8}$) is represented by a red line and suggestive threshold ($P<1x10^{-5}$) is represented by a blue line.

The top four loci for each GWAS below a *P* value of $5x10^{-7}$ for the treatment resistance meta-analysis and stages of resistance can be found in **Table 4.3**. Heterogeneity statistics for the treatment resistance meta-analysis are also reported, we found either no evidence or nominal evidence of heterogeneity between GS:SFHS and GENDEP that was not significant after adjustment for multiple correction at a genome-wide level.

Treatment Resistance (Meta-analysis)											
SNP	**Chr**	**P value**	**OR (CI)**	**Average Freq**	**Alleles**	**Locus**	**Gene or (closest)**	**GS:SFHS OR (CI)**	**GENDEP OR (CI)**	**Heterogeneity I^2**	**Heterogeneity *P* value**
rs188352979	10	$3.3x10^{-7}$	2.87 (2.47-3.28)	0.017	A/G	Intergenic	(ACADSB – HMX3)	3.33 (2.90 – 3.76)	1.33 (0.31 – 2.35)	62.0	0.10
rs145842949	16	$3.9x10^{-7}$	2.42 (2.08-2.77)	0.030	T/G	Intergenic	(MON1B)	2.35 (1.96 – 2.74)	2.69 (1.96 – 3.42)	0.0	0.75
rs111968111	6	$3.9x10^{-7}$	3.00 (2.58-3.42)	0.014	T/C	Intergenic	(SRPK1 - SLC26A8)	3.53 (3.08 – 3.98)	0.84 (-0.42 – 2.10)	77.5	0.035
rs138583130	12	$5.0x10^{-7}$	2.50 (0.1894)	0.023	A/C	Intergenic	(CNTN1)	2.57 (2.16 – 2.98)	2.72 (1.75 – 3.68)	0.0	0.92

Stages of Resistance (GS:SFHS)								[redacted]
SNP	**Chr**	**P value**	**Beta (SE)**	**Freq**	**Alleles**	**Locus**	**Gene or (closest)**	
rs116902282	10	$1.5x10^{-7}$	0.49 (0.076)	0.011	T/C	Intergenic	(C10orf35 – COL13A1)	
rs188352979	10	$4.0x10^{-7}$	0.33 (0.065)	0.016	A/G	Intergenic	(ACADSB – HMX3)	
rs182041872	10	$5.2x10^{-7}$	0.30 (0.060)	0.019	G/A	Intergenic	(ACADSB – HMX3)	
rs4400118	5	$5.4x10^{-7}$	0.29 (0.058)	0.020	G/A	Intergenic	(DNAH5 – TRIO)	

4.3. Top GWAS loci in treatment resistance (TR) and stages of resistance (SR). Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; MAF, minor allele frequency; CI, confidence interval; SE, standard error; GS:SFHS, Generation Scotland: Scottish Family Health Study; GENDEP, Genome-based Therapeutic Drugs for Depression.

Gene and gene-set enrichment analysis

Gene-based analysis with MAGMA did not identify any genes significantly associated after false discovery rate (FDR) multiple testing correction. Similarly, in the gene-set analysis no gene-set passed multiple testing correction over 10,000 permutations. The most significant genes and gene-sets are listed in **Supplemental Table S4**.

Pedigree-based heritability and genetic correlations

Pedigree-based heritability was calculated in GS:SFHS at 0.60 (CI=0.22-0.87) for treatment resistance and 0.27 (CI=0.24-0.31) for stages of resistance. Genetic correlation between treatment resistance and stages of resistance was calculated at 0.90 (P=0.0034).

Significant positive genetic correlations were found between treatment resistance and neuroticism (r_g=0.66, P_{FDR}=0.0091), MDQ (r_g=0.86, P_{FDR}=0.0072) and GHQ (r_g=0.96, P_{FDR}=8.8x10^{-5}). For stages of resistance they were identified between neuroticism (r_g=0.51, P_{FDR}=0.023), SPQ (r_g=0.44, P_{FDR}=0.036), MDQ (r_g=0.69, P_{FDR}=0.027) and GHQ (r_g=0.71, P_{FDR}=0.0011). All these correlations survived correction for multiple testing with FDR (**Table 4.4**).

	Treatment Resistance				Stages of Resistance			
	P value	**P_{FDR}**	**r_g (s.e.)**	**N**	**P value**	**P_{FDR}**	**r_g (s.e.)**	**N**
Neuroticism	**0.0034**	**0.0091**	**0.66 (0.26)**	**3,133**	**0.0058**	**0.023**	**0.51 (0.19)**	**3,133**
Extraversion	0.78	0.78	-0.059 (0.22)	3,133	0.71	0.71	-0.063 (0.17)	3,133
SPQ	0.070	0.11	0.43 (0.26)	1,607	**0.018**	**0.036**	**0.44 (0.20)**	**1,607**
MDQ	**0.0018**	**0.0072**	**0.86 (0.36)**	**1,715**	**0.010**	**0.027**	**0.69 (0.28)**	**1,715**
GHQ	**$1.1x10^{-5}$**	**$8.8x10^{-5}$**	**0.96 (0.26)**	**3,378**	**0.00014**	**0.0011**	**0.71 (0.19)**	**3,378**
'g'	0.32	0.37	-0.16 (0.17)	3,349	0.067	0.091	-0.24 (0.133)	3,349
Education	0.13	0.17	-0.33 (0.23)	3,233	0.40	0.46	-0.15 (0.18)	3,233
SIMD	0.063	0.11	-0.22 (013)	3,268	0.068	0.091	-0.18 (0.099)	3,268

4.4. Genetic correlations of treatment resistance and stages of resistance in Generation Scotland: Scottish Family Health Study with cognitive and personality traits. Significant values after multiple testing correction (P_{FDR}<0.05) are shown in bold. Abbreviations: FDR, false discovery rate; rP, phenotypic correlation; SPQ, schizotypal personality questionnaire; MDQ, mood disorder questionnaire; GHQ, general health questionnaire; SIMD, Scottish index of multiple deprivation.

Treatment resistance and stages of resistance were positively and nominally associated with MDD PRS at *P* value thresholds (P_T) 0.1, 0.5 and 1, and treatment resistance only with schizophrenia PRS at P_T=0.01. There were no significant associations between treatment resistance or stages of resistance with bipolar disorder PRS (**Table 4.5**). No result survived FDR correction and power analyses indicated we were underpowered to detect an association between MDD and bipolar disorder PRS with treatment resistance and stages of resistance. Schizophrenia PRS was powered to detect an association at all thresholds in both treatment resistance and stages of resistance given a genetic correlation of 0.5. At a genetic correlation of 0.25, stages of resistance had adequate power at all thresholds whilst treatment resistance was only powered at P_T 0.01 (**Table 4.5**).

		Treatment Resistance				Stages of Resistance			
	Threshold	P_{FDR}	Beta	R^2	Power at r_g 0.5 (& 0.25)	P_{FDR}	Beta	R^2	Power at r_g 0.5 (& 0.25)
MDD	*0.01*	0.54	0.25	0.00030	0.17 (0.079)	0.57	0.0055	0.00021	0.44 (0.15)
	0.05	0.46	0.0058	0.00050	0.17 (0.080)	0.46	0.0086	0.00051	0.45 (0.15)
	0.1	0.21	0.0017	0.0014	0.17 (0.079)	0.25	0.014	0.0013	0.44 (0.15)
	0.5	0.20	0.012	0.0020	0.16 (0.076)	0.15	0.017	0.0020	0.41 (0.14)
	1	0.20	0.060	0.0015	0.16 (0.076)	0.15	0.0160	0.0018	0.40 (0.14)
SCZ	*0.01*	0.20	0.011	0.0017	1.0 (0.85)	0.36	0.010	0.00074	1.0 (1.0)
	0.05	0.46	0.0061	0.00055	1.0 (0.75)	0.74	0.0032	$7.1x10^{-5}$	1.0 (1.0)
	0.1	0.68	0.0019	$5.3x10^{-5}$	1.0 (0.68)	0.87	0.0011	$8.1x10^{-6}$	1.0 (0.99)
	0.5	0.56	0.0038	0.00021	0.98 (0.52)	0.60	0.0047	0.00015	1.0 (0.95)
	1	0.56	0.0036	0.00020	0.98 (0.50)	0.60	0.0050	0.00018	1.0 (0.94)
BPD	*0.01*	0.65	-0.0025	$9.6x10^{-5}$	0.25 (0.099)	0.75	0.0026	$4.7x10^{-5}$	0.64 (0.21)
	0.05	0.65	0.0024	$8.6x10^{-5}$	0.26 (0.10)	0.60	0.0050	0.00017	0.65 (0.22)
	0.1	0.56	0.0034	0.00017	0.25 (0.099)	0.48	0.0074	0.00038	0.65 (0.22)
	0.5	0.46	0.0053	0.00042	0.24 (0.095)	0.36	0.010	0.00074	0.61 (0.20)
	1	0.46	0.0053	0.00041	0.23 (0.095)	0.36	0.0097	0.00066	0.60 (0.20)

4.5. PRS associations with schizophrenia, bipolar disorder and MDD with treatment resistance and stages of resistance . Abbreviations: MDD, major depressive disorder; SCZ, schizophrenia; BPD; bipolar disorder, r_g; genetic correlation.

4.2.5. Discussion

We utilised antidepressant prescription records to explore common genetic factors in treatment resistance and stages of resistance in a population and family-based cohort of 3,452 individuals. In the treatment resistance GWAS meta-analysis, the most significant locus was located at 10q26.13 at $P= 3.3x10^{-7}$; lead SNP rs188352979. This SNP is intergenic and lies in between genes *ACADSB* and *HMX3*. *ACADSB* encodes short/branched chain specific acyl-CoA dehydrogenase (SBCAD) which is an enzyme involved in the metabolism of fatty acids (Rozen et al., 1994). *HMX3* is a transcription factor that is involved in the specification of neuronal cells needed for hypothalamus development and hypothalamic-pituitary-adrenal (HPA) axis (Wang, Grimmer, Van De Water, & Lufkin, 2004). Disruptions in the HPA axis are known to be associated with MDD, MDD severity and antidepressant response (Papiol et al., 2007; Pariante & Lightman, 2008). A single locus at 10q22.1 was associated with SR at $P=1.71x10^{-7}$, lead SNP rs116902282. This is an intergenic variant that lies between functional genes *C10orf35* and *COL13A1*. *C10orf35* is a protein coding gene that has previously been associated with uterine leiomyoma (Ling, Wu, Fu, Tan, & Xu, 2015). *COL13A1* encodes the alpha chain of one of the nonfibrillar collagens. No variant in either analysis reached the required threshold for genome-wide statistical significance ($P>5x10^{-8}$). Gene and gene-set enrichment did not identify any significant associations with either treatment resistance or stages of resistance. Nonetheless, modest to high pedigree-based heritability estimates indicate that 60% of the variance in treatment resistance and 27% of the variance in stages of resistance can be explained by genetics, although these estimates had large confidence intervals. This indicates that further exploration into genetic contributions in antidepressant resistance may be beneficial.

The genetic correlation between treatment resistance and stages of resistance (r_g=0.90) demonstrates that these two measures capture similar genetic variation. This is further shown in the similarity of the genetic correlation measures with cognitive and behavioural measures, albeit stages of resistance is a quantitative trait and is therefore higher powered in this analysis. Treatment resistance was significantly and positively genetically correlated with neuroticism (r_g=0.66), MDQ (r_g=0.86) and GHQ (r_g=0.96) indicating overlapping genetic architecture between these traits. In stages of resistance,

these same traits also demonstrated significant genetic correlation; neuroticism (r_g=0.51), MDQ (r_g=0.69) and GHQ (r_g=0.71), and we additionally identified a significant genetic correlation with SPQ (r_g=0.44). The MDQ and SPQ correlation indicates that more resistant individuals may share genetic components with schizotypy and mood disorder personality traits. Genetic overlap between psychological distress and antidepressant resistance is indicative that individuals susceptible to distress are associated with a poorer outcome in antidepressants and should be further investigated. We did not find any correlation in treatment treatment resistance or stages of resistance with general intelligence, education or social deprivation trait, however, a lack of power due to small sample size (n=3,452 with only 250 cases in treatment resistance) was likely to be a major contributing factor. We have only identified modest to high correlations therefore it may be possible that we are underpowered to detect weak to modest correlations. It is also worth noting that whilst these traits were measured prior to the treatment resistance definition, individuals may have been diagnosed with depression and/or taking antidepressants prior to this as we only have prescription data from 2009. Therefore, it is not possible to determine cause or effect in these associations. These phenotypes have also been previously associated with depression severity, e.g. higher psychological distress is associated with higher depressive symptoms (Marchand, Durand, Juster, & Lupien, 2014). However, studies on treatment resistance and MDD have been largely contradictory. Whilst some have found that higher severity is associated with treatment resistance (Souery et al., 2007) others have found treatment responders are associated with clinical severity (Fournier et al., 2010). Additionally, some studies have found no association between the two (Rabinowitz et al., 2016).

Using PRS, we investigated whether poor response to antidepressants indicate a higher liability to other mental disorders (schizophrenia or bipolar disorder) as well as higher genetic loading of MDD itself. We did not find any significant association with treatment resistance or stages of resistance, however, power with the current sample size was only adequate in analysis with schizophrenia PRS at a genetic correlation of 0.5 and additionally 0.25 for stages of resistance. Results for MDD and bipolar disorder PRS should therefore be treated with caution, although the nominal associations between MDD PRS and stages of resistance may be worth further

investigation with larger sample sizes.

One of the strengths of this study was that it used data taken from a population-based cohort and is a good representation of antidepressant users in a MDD sample in the general Scottish population. Nevertheless, certain limitations of this study should be noted. Although currently diagnosed schizophrenia and bipolar patients were removed prior to this analysis and minimum dose was matched to that of MDD recommendations, it is possible that individuals were prescribed the antidepressant for other conditions e.g. anxiety disorders, obsessive compulsive disorder (OCD), posttraumatic stress disorder (PTSD) and panic disorders, and may have had a misdiagnosis in the first instance. It was not possible to account for the use of psychotherapy or electroconvulsive therapy (ECT; which is advised in patients with severe MDD). We were also only able to obtain prescription records over a six-year period meaning there are likely to be some individuals who had prescriptions before this period. Furthermore, because we integrate prescribing data across a number of different antidepressant drugs and classes, specific associations with treatment resistance within or between classes of prescribed compounds may have been missed. In the meta-analysis, it should also be noted that the samples had differing covariates, e.g. baseline severity in the GENDEP sample but not GS:SFHS. Prescription records are also susceptible to low drug adherence which are known to be a problem in antidepressant treatment. Specifically, 20% of treatment resistant individuals are thought to represent non-compliant individuals (Souery & Mendlewicz, 1998) and it is therefore possible that low drug concordance is contributing to an individual's treatment resistance status and therefore is a notable limitation. Additionally, the cross-sectional nature of the treatment resistance definition in GS:SFHS means that any trait association is subject to change throughout the time-period that the definition was constructed. Moreover, it is difficult to assess cause or effect given that treatment resistant individuals may have already failed to respond to two antidepressants prior the definition construction.

Despite an increased sample size compared to those reported in previous clinical studies of antidepressant response (n=2,897), our numbers are still small for a GWAS and it is likely that we were underpowered. With an increasing availability of electronic records in large biobanks and numerous smaller antidepressant studies, a

collaborative effort approach may be required in order to increase sample size for adequate power. To replicate our analysis with adequate power (>0.8) at a MAF of 0.01, it would require a sample size of 7,596 cases for treatment resistance and 9,660 total sample for stages of resistance assuming an OR of 1.6 and beta of 0.3, respectively (**Supplemental Table S6**; power calculations were completed using QUANTO v1.2).

With increasing accessibility of electronic health records (Jensen, Jensen, & Brunak, 2012), access to prescription records is becoming possible. In this study, we explored the possibility of utilising this prescription data to examine resistance to antidepressant treatment by inferring drug switching as non-response. We have provided evidence that resistant individuals have a high genetic correlation with neuroticism, psychological distress, schizotypy and mood disorder traits. Furthermore, we demonstrate the need for larger cohorts and collaboration in order to maximise sample size. This study demonstrates the value of this method; as larger cohort sizes become available, the results of such studies could further inform clinical and research applications.

Acknowledgments

This investigation was supported by the Wellcome Trust 104036/Z/14/Z (STRADL, Stratifying Resilience and Depression Longitudinally). Generation Scotland received core funding from the Chief Scientist Office of the Scottish Government Health Directorate CZD/16/6 and the Scottish Funding Council HR03006. We thank all families, practitioners and the Scottish School of Primary Care involved in the recruitment process as well as the entirety of Generation Scotland team; interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, healthcare assistants and nurses. We are grateful to the Sackler Foundation for the generous support of this work. IJD is supported by MRC and BBSRC funding to the University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology (MR/K026992/1). This report represents independent research part-funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's

College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

Conflict of Interest

AMM has received financial support from Pfizer (formerly Wyeth), Janssen and Lilly. The remaining authors declare no conflict of interest.

4.3. Chapter Conclusion

Although this current analysis is relatively small for a GWAS, it demonstrates the potential utility of population-based cohorts and electronic health records for pharmacogenomic studies. In this study, we demonstrate that 7,596 treatment resistance cases would be necessary to be adequately powered to detect a genetic variant with an OR= 1.6 and a MAF= 1% whereas this study was underpowered with 358. The emerging large national biobanks, such as UK Biobank, represent examples whereby sample sizes could overcome current power issues. Additionally, meta-analysis between existing cohorts and these biobanks could also be applied in order to achieve well-powered genome-wide analysis that more accurately reflects the effect of common variants.

Although common variants have been demonstrated to contribute a proportion of the variance in antidepressant response (GENDEP Investigators et al., 2013), it may also be advantageous to examine rare variants. A 2013 analysis examined exome sequencing data in two samples of patients taking escitalopram (n=116 and n=394) identifying a variant in the *BMP5* gene associated with worse treatment response (Tammiste et al., 2013). Nonetheless, the sample here was small and association analysis of rare variants typically requires large sample sizes (B. Li & Leal, 2008), arguably greater than those required for a GWAS (Sham & Purcell, 2014). Furthermore, it has been reported that women preferentially respond to SSRIs in comparison to men, (Berlanga & Flores-Ramos, 2006; Kornstein et al., 2000) therefore, given the availability of adequately sized samples, gender-stratified GWAS may further elucidate this.

The genetic correlations identified here, indicate that these traits may be of interest as potential intermediate phenotypes in antidepressant treatment resistance, therefore, further evidence and specific intermediate phenotype exploration may be of interest to confirm this. For example, it is possible that treatment resistant depression represents a subgroup of depression with higher neuroticism that may play a biological role in non-response. Intermediate phenotypes have been described in multiple psychiatric conditions including MDD, schizophrenia and alcoholism (Hornung & Heim, 2014; Oreland et al., 2017; Tan, Callicott, & Weinberger, 2008)

and have been suggested to be effective tool in identification of genetic subgroups in psychiatric disorders (Flint et al., 2014). Genetic overlap between two phenotypes indicates that the phenotypes may have an important causal relationship, although further research is needed to confirm this. Efforts in antidepressant treatment resistance have, for the most part, focused on monoaminergic candidate genes (Leuchter et al., 2014). For instance, the serotonin transporter (*5-HTTLPR*) is related to the amygdala-cingulate feedback circuit involved in emotion regulation (Pezawas et al., 2005) and appears to modulate amygdala responses to negative stimuli (Dannlowski et al., 2010). Additionally, the noradrenaline transporter (*NET*) and *5-HT_{1A}* polymorphisms have been associated with hippocampal volume in treatment resistant individuals (J. L. Phillips et al., 2015). Nevertheless, candidate gene-studies are prone to selection bias (bias introduced by hypothesis-driven selection of a gene) and associations between antidepressant response monoaminergic genes are poorly replicated (Fabbri et al., 2014). An alternative approach would be to use summary data from GWAS to measure genetic correlations in common variants (as in Chapter 3). However, even with the increase in numbers we report here, numbers are currently too small to run these using current LDSC techniques (which typically require a minimum of 5,000 individuals). In this study, we demonstrate pedigree-based genetic correlations, which account for all genetic variance including common variants, rare variants and gene interactions, therefore providing evidence for intermediate phenotypes between genes and treatment resistant outcome. Nevertheless, this approach is prone to overestimation due to shared environment (Manolio et al., 2009). Should interindividual variability in antidepressant response represent clinical depression subgroups, further explication of intermediate phenotypes may prove valuable.

Chapter 5. Non-genetic Risk Factors of Antidepressant Treatment Resistance

5.1. Background

In Chapter 4, neuroticism was identified as a potential intermediate phenotype whereby higher neuroticism may mediate the effect of risk alleles on antidepressant treatment resistance. The role of neuroticism as a genetic intermediate phenotype has previously been reported in MDD. Neuroticism polygenic risk score (PRS) has demonstrated a positive association with MDD status (de Moor et al., 2015) and neuroticism and MDD were positively genetically correlated (rg= 0.64) in an analysis conducted using linkage disequilibrium score regression (LDSC) (D. J. Smith et al., 2016). Therefore, it may be of value to further explicate the mechanism by which neuroticism is related to antidepressant resistance**.**

The role of stress is well-established in MDD and is estimated to precede a depressive episode in roughly 70% of cases as well as play a causal role in 20-50% of cases (Hammen, 2005; Monroe & Harkness, 2005). Stress is implicated in most causal explanations for MDD, for example hypothalamus-pituitary-adrenal (HPA) axis dysregulation (Belmaker & Agam, 2008), inflammatory mechanisms (Dantzer et al., 2008) and the brain-derived neurotrophic factor (BDNF) hypothesis (Belmaker & Agam, 2008), amongst others (covered in more detail in Chapter 1). However, there is currently no steadfast explanation for the variability in mental health outcome after a stressful experience. It has been theorised that personality traits, such as neuroticism, can influence response to stress which, in turn, can induce MDD (Yoon, Maltby, & Joormann, 2013). Neuroticism is one of five personality constructs that form the five-factor model, which also include extraversion, conscientiousness, agreeableness and openness to experience (Goldberg, 1993) but is arguably the personality trait that is most widely associated with negative mental health outcomes. Specifically, it has been shown to positively associate with MDD (Kendler, Kuhn, & Prescott, 2004), higher stress vulnerability (Schneider, 2004) and negatively associate with subjective well-being (Steel, Schmidt, & Shultz, 2008). Higher levels of neuroticism has been shown

to increase perceived stress (Gramstad, Gjestad, & Haver, 2013) and encourage less adaptive stress coping strategies, such as increased emotional and avoidant/escape-focused coping and reduced task/problem-focused coping (Bolger, 1990; Watson & Hubbard, 1996).

Many factors are known to affect responses to stress and the term resilience is often used as a general term to describe an ability to bounce back from adversity, although there is no one universally accepted definition (Southwick & Charney, 2012). Higher resilience is thought to protect individuals from MDD and other mental health outcomes (Wu et al., 2013). Specifically, it has been found that a higher resilience reduces the severity of depression after a traumatic event (Wingo et al., 2010). Resilience is a construct that comprising of many factors including coping style in response to stress. Maladaptive coping styles (such as emotional coping) have been associated with an increased risk for MDD (Horwitz, Hill, & King, 2011). Coping style has been shown not only to predict later depression but also to be predictive of depressive symptoms (Murberg & Bru, 2005). Moreover, coping styles are thought to moderate response to stressful life events; for instance, it has been found that depression can lead to a poor assessment of coping which further leads to the sufferer feeling overwhelmed in stressful situations (Orzechowska, Zajączkowska, Talarowska, & Gałecki, 2013). It is possible that all these factors could independently impact MDD or interact to elicit the disorder.

Models to explore the relationships between neuroticism, stress and MDD have primarily focused on mediation and moderation studies which, in larger samples, are sometimes separated by gender on account of neuroticism being higher in women (Weisberg, Deyoung, & Hirsh, 2011). For instance, the pathway between neuroticism and MDD was found to be mediated by stress in a sample of 3,950 individuals, whereby neuroticism increased stress which then induced depressive symptoms (male coefficient= -0.071, $P<0.001$, female coefficient= -0.039, $P<0.001$). However, neuroticism was also directly associated in the same model (male coefficient= 0.077, $P<0.001$, female coefficient= 0.077, $P<0.001$), meaning the mediation was only partial and it was associated with the outcome both directly and indirectly (S. E. Kim et al., 2016). Moreover, neuroticism has also been shown to moderate the relationship

between cumulative stress with depressive symptoms in a sample from the general population (n= 563) and a replication clinical depression and anxiety study sample (n= 2,274), in which the combined effect of cumulative stress and neuroticism strengthened the association with MDD (discovery beta= 0.013, *P*<0.001, replication beta = 0.367, *P*<0.001) (Vinkers et al., 2014). Maladaptive coping styles (such as emotional coping) have also been shown to mediate the relationship between neuroticism and MDD in a sample of 533 individuals (pathway from neuroticism to maladaptive coping: beta= 0.64, *P*<0.001; pathway from maladaptive coping to depression: beta= 0.61, *P*<0.001) (Yoon et al., 2013) indicating a possible pathway by which neuroticism can induce stress vulnerability and then depressive outcome.

Higher levels of both neuroticism and childhood maltreatment have also been shown to predict poorer response to antidepressants (Bock, Bukh, Vinberg, Gether, & Kessing, 2010; Nanni et al., 2012; Quilty, Meusel, & Bagby, 2008), yet their interrelationships have not yet been explored. The largest analysis to date examining neuroticism in antidepressant responder and non-responders was conducted on 649 individuals and reported significantly higher neuroticism in non-responders (t = 13.89, *P*< .01). As neuroticism has been associated with both MDD and antidepressant non-response, it is possible that perhaps treatment resistant individuals represent a subgroup of depressed individuals with a higher level of neuroticism. Consequently, a different approach to such individuals may elicit more effective treatment outcomes. Furthermore, explicating the pathways from neuroticism to outcome may help elucidate more proximal processes involved in antidepressant treatment resistance.

This chapter will explore the associations between antidepressant treatment resistance and neuroticism, psychological resilience and coping styles as well as the interrelationships between them. The content for this chapter has been summarised in a manuscript entitled “Antidepressant treatment resistance and the impact of neuroticism, psychological resilience and coping style” which has been submitted for publication. I confirm, as the first author, that I carried out the analysis of the data and wrote the following paper.

5.2. Antidepressant Treatment Resistance and the Impact of Neuroticism, Psychological Resilience and Coping Style

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Number of words (main text)	3,352
Number of references	53
Number of tables	3
Number of figures	2

5.2.1. Abstract

Background: Antidepressant response rates are modest with only 50% of individuals achieving symptomatic remission after two adequate treatment trials. Higher neuroticism is associated with poor response to antidepressants but the role of psychological resilience and coping styles have been incompletely studied. This study examined neuroticism, psychological resilience and coping styles in antidepressant resistance and their potential mediating and moderating effects.

Methods: Using prescribing records in the Generation Scotland cohort, antidepressant treatment resistance (TR) was defined as non-response to two adequate treatment trials whereby switching to a different antidepressant was a proxy for non-response. The independent effects of neuroticism, psychological resilience and coping styles (task-orientated, emotion-orientated, and avoidance-orientated coping) on TR were examined in addition to their moderation and mediation effects.

Results: Neuroticism and emotion-orientated coping were independently positively associated with TR whereas psychological resilience, task-orientated and avoidance-orientated coping were negatively associated. No moderation effect of resilience or coping style on neuroticism was identified. Mediation analysis suggested that the positive association between neuroticism and TR is partially mediated by lower resilience, although no effect of task, emotion or avoidance-orientated coping was found.

Conclusions: Neuroticism, lower psychological resilience and less adaptive coping styles are each associated with TR. The effects of coping styles in TR present potentially modifiable risk factors. In addition to the stress-ameliorating effects of psychological resilience observed for the onset of MDD, our research shows that these effects may generalise to better treatment outcomes in people previously affected by depression.

5.2.2. Introduction

Antidepressants are the main therapeutic treatment for major depressive disorder (MDD); however, response rates are modest with only a third of individuals in remission after completion of one adequate antidepressant trial (M. H. Trivedi et al., 2006) and half after the completion of two (A. Rush et al., 2006). Early improvement in MDD symptomatology whilst taking an antidepressant has been demonstrated to predict subsequent remission (Lam, 2012). Nonetheless, the mechanisms of depression and antidepressant resistance remain poorly understood. Psychological factors affecting treatment response might represent potentially modifiable influences that have so far received relatively little attention in the published literature.

Antidepressant treatment resistance (TR) is commonly defined as failure to respond to more than two treatment trials given adequate dose and duration (Conway et al., 2016). Determining the potentially modifiable psychological factors conferring TR may have important benefits for the patient's health, their families and society. Several studies have employed multiple sociodemographic and clinical factors to construct prediction models for TR (Chekroud et al., 2016; Kautzky et al., 2017; Perlis, 2013). Most antidepressants act through the monoaminergic system and increase neurotransmitter levels such as serotonin. Neuroticism has been shown to positively associate with the higher serotonin transporter binding (Takano et al., 2007), the target of selective serotonin reuptake inhibitors (SSRIs). Higher neuroticism has been associated with risk for MDD (Kendler et al., 2004; Lyness, Duberstein, King, Cox, & Caine, 1998) and poor treatment outcomes in both first and second antidepressant trials (Bock et al., 2010; Quilty, De Fruyt, et al., 2008). Moreover, greater reduction in neuroticism score has been shown to significantly mediate clinical response to SSRIs (Quilty, Meusel, et al., 2008). Although Quilty *et al.*, (2008b) demonstrated the mediation was specific to SSRIs, it indicates that clinical response to antidepressants may be secondary to reductions in neuroticism.

Psychological resilience is generally considered to be the ability to recover or 'bounce back' from stress. Lower resilience is associated with affective disorders including MDD (T. Hu, Zhang, & Wang, 2015) and has been shown to negatively associate with neuroticism (Campbell-Sills, Cohan, & Stein, 2006). Furthermore, resilience has been

demonstrated to mediate the effects of neuroticism on happiness and positive affect (Wei, Wang, Liu, & Zhang, 2014). Early symptom improvement has been used as a proxy for resilience to MDD, with findings suggesting that it is associated with subsequent treatment outcome (Wagner et al., 2017). However, psychological resilience has been little studied with regard to TR, which is likely due to the broadness of its definition and complexities in its measurement (Windle, Bennett, & Noyes, 2011). As yet, no study has investigated whether a quantitative measure of resilience is associated with TR.

Personality traits have been demonstrated to predispose an individual towards a particular coping style, specifically, higher neuroticism has been shown to increase the risk for unhealthy coping styles (Drapeau, Cerel, & Moore, 2016). This can in turn increase liability to stress (Afshar et al., 2015) which has been associated with a reduced hippocampal neurogenesis that is known to be reversed by antidepressants (Warner-Schmidt & Duman, 2006). Despite the known effect of neuroticism on antidepressant response, no study to date has explored the interrelationships between neuroticism, psychological resilience and coping style in TR, to our knowledge. It is both possible that the association between high neuroticism and treatment resistance occurs via resilience or coping style pathways (mediation) or that higher resilience or different coping styles reduce the impact of high neuroticism on treatment resistance and vice versa (moderation). Neuroticism is generally considered to remain relatively stable in adulthood (Lucas & Donnellan, 2011), although in the presence of depression and anxiety it has been demonstrated to be subject to small changes (Karsten et al., 2012). In this present analysis, we will treat neuroticism as stable trait.

In this study, we explore neuroticism, resilience (measured by the Brief Resilience Scale) and coping styles (task, emotional and avoidance-orientated) in association with TR in a large population-based cohort (Generation Scotland: Scottish Family Health Study; GS:SFHS). Using logistic regression, we examined the independent association and moderating effects of resilience and coping styles on the association between neuroticism and TR. We also used structural equation modelling to examine the potential mediating relationship of the aforementioned variables on neuroticism and TR.

5.2.3. Methods

Cohort Description and genotyping

Generation Scotland: Scottish Family Health Study

GS:SFHS is a family and population-based cohort recruited at random from Scottish medical practices between 2006-2011. At baseline, GS:SFHS recruited 24,090 individuals (mean age= 47.6, s.d.= 15.4) to the study and conducted a battery of mental health, cognitive, psychosocial and biological testing. In 2014, participants were re-contacted and asked to participate in a follow-up study examining mental health and resilience. A total of 9,618 individuals (mean age= 56.4, s.d.= 13.3) responded to follow-up and provided self-report measures of stressful life events, resilience and coping styles. Full details of the initial recruitment and follow-up have been given elsewhere (Navrady et al., 2017; B. Smith et al., 2006; B. H. Smith et al., 2013) and further information is provided in the Supplementary Materials.

Defining Treatment Resistance

Prescription data was available through linkage to the Prescribing Information System (PIS) (Alvarez-Madrazo, McTaggart, Nangle, Nicholson, & Bennie, 2016) administered by National Health Service (NHS) Scotland Information Services Division. PIS is a database of all Scottish NHS prescriptions for payments for medications excluding hospital dispensed prescriptions and over-the-counter medications (Prescription indication was not included and prescription records were available between February 2009 and April 2015, see Supplementary Materials). 98% gave consent for record linkage at the initial interview. To measure TR, all antidepressant users were extracted (frequency table in **Supplementary Table S1**) and thresholds of dose and duration applied. Daily dose was calculated for each prescription entry and all those that fell below British National Formulary (BNF) (Joint Formulary Committee, 2017) recommendations for MDD were removed, as this is the medication reference book for the NHS. Duration of antidepressant use was also calculated and prescriptions that fell below 6 weeks were removed (see Supplementary Materials) giving a total of 3,839 individuals taking antidepressants in GS:SFHS. Individuals with schizophrenia, schizoaffective disorder and bipolar disorder were

excluded (n=164). TR cases were defined as individuals who had been prescribed more than two antidepressants and controls were individuals who had been prescribed one or two antidepressants. A sample of unrelated individuals was used for all analysis therefore a total of 2,390 controls and 262 cases were available.

Neuroticism, resilience and coping style variables

Neuroticism was measured at initial clinic visit using the Eysenck Personality Questionnaire Short Form-Revised (EPQ-SF) (Eysenck, 1991). The neuroticism subsection consisted of 12 items which were answered "no" or "yes" (0 and 1, respectively), therefore individuals received a score ranging from 0 to 12 with higher scores indicating higher neuroticism (mean=3.8, total n=19,758).

Resilience was measured at follow-up using the Brief Resilience Scale (BRS) (B. W. Smith et al., 2008); a self-report measure assessing an individual's ability to recover from stress. The BRS consists of six statements (e.g. "I tend to bounce back quickly after hard times") rated on a 5-point scale from "Strongly agree" to "Strongly disagree". The BRS was converted to a numeric 1 to 5 scale, with reverse scoring of even-numbered items, and the average of the sum of the six questions was taken (mean=3.6, total n=9,411). The BRS has previously been found to demonstrate good internal consistency and test-retest reliability (B. W. Smith et al., 2008).

Coping styles were measured at follow-up by the Coping Inventory for Stressful Situations (CISS) (Cosway, Endler, Sadler, & Deary, 2000; Endler & Parker, 1990) which consists of a list of 48 statements (e.g. "Schedule my time better") grouped into three overarching coping styles; task-orientated coping, emotion-orientated coping and avoidance-orientated coping. Individuals ranked each in terms of relevance to themselves in situations when they are under stress from 1 ("Not at all") to 5 ("Very much"). The scores for each coping style sub-scale were then summed (task-orientated: mean=54.3, total n=8,980, emotion-orientated: mean=37.6, total n=9,130 and avoidance-orientated: mean=39.4, total n=9,070). The CISS has proven a robust measure of assessing situation-specific coping strategies, with a stable factor structure and high construct validity (Endler & Parker, 1990).

It should be drawn to the readers attention that the neuroticism was measured between 2006-2011, treatment resistance was defined between 2009-2015 and coping styles were measured between 2014-2017. This should therefore be considered an important limitation in the following analysis.

Statistical Analysis

Independent associations

Univariate analysis of TR was performed using logistic regression in generalised linear models in R version 3.2.3 (www.r-project.org). TR was fitted as a binary outcome in all models and neuroticism, BRS, task-orientated, emotion-orientated and avoidance-orientated coping were fitted as the independent predictors. As TR was taken over a ~ 6-year time period and all independent variables taken at a single time point, independent variables were adjusted for age at measurement before being fitted to the model, and TR was adjusted for current age and sex. Variance explained was calculated using McFadden pseudo R^2. Results were corrected for multiple testing using false discovery rate (FDR) over the five models.

Moderation

Bivariate generalised linear models were computed to examine moderating effects and possible interactions. TR was fitted as a dichotomous outcome and neuroticism was tested for interactions with BRS, neuroticism, task-orientated, emotion-orientated and avoidance-orientated coping. The main effect of each variable in addition to their interaction was investigated. As above, predictor variables were adjusted for age at measurement prior to being modelled and TR was covaried for current age and sex.

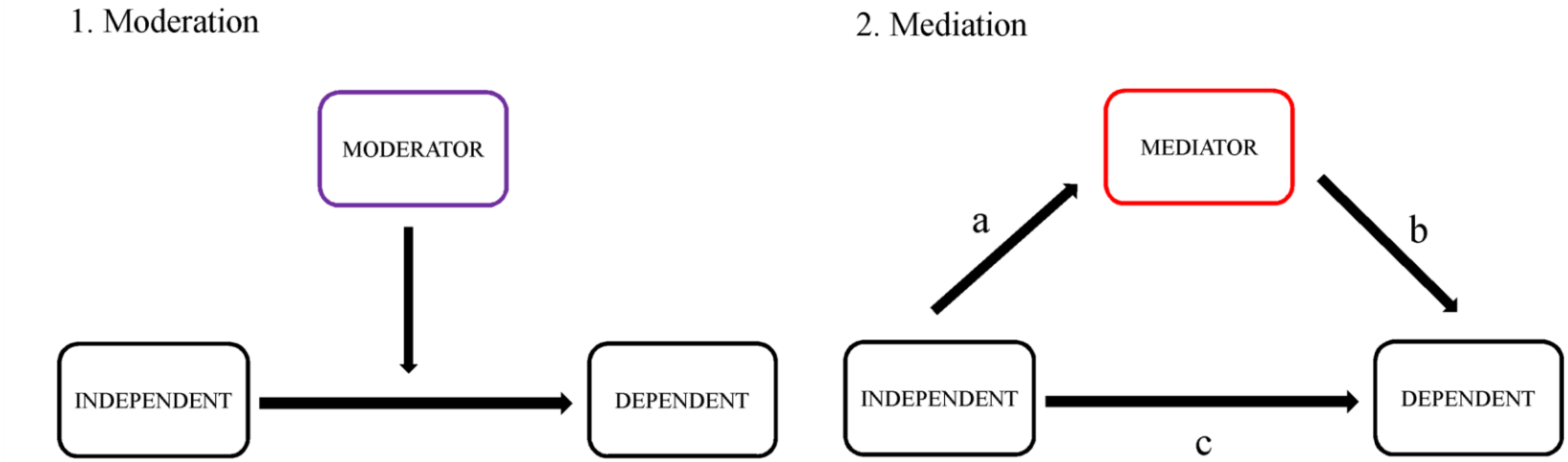
Full model with treatment resistant outcome

The variables were assessed in a full model to assess their association as independent variables with TR outcome. Due to the co-linearity of the three coping styles these were assessed in three separate models. This analysis was completed using the structural equation modelling package 'lavaan' (Rosseel, 2012) in R using the WLSMV (weighted least squares, mean and variance adjusted) estimation and standardised betas are reported in the model. Neuroticism, resilience and each coping

style were assessed as independent predictors of TR and the correlations between each variable is reported.

Mediation

Mediational analyses were completed using the structural equation modelling package 'lavaan' (Rosseel, 2012) in R. WLSMV estimation was used to model the binary TR outcome in all analyses; standardised betas were reported throughout. For each model, the direct association between TR and neuroticism (path c) was examined and the mediating associations between neuroticism and resilience or coping style (path a) and then resilience or coping style and TR (path b) were examined independently in each model (**Figure 5.1**). Significant a and b paths suggest significant model mediation. BRS and coping style variables were measured as latent constructs, with the variance of each fixed to 1 for comparability of the models. Fit statistics to determine the fit of each model are reported; comparative fit index (CFI; good fit > 0.95), Tucker-Lewis index (TLI; good fit > 0.95) and root mean square error of approximation (RMSEA; good fit < 0.06) (L. Hu & Bentler, 1999).



5.1. Diagram represeenting the moderation and mediation models and paths. The image represents (1) mediation and (2) moderation whereby the letter c represents the direct pathway and a and b the indirect pathway.

5.2.4. Results

A Pearson's correlation matrix between TR, neuroticism, resilience and coping styles on complete data (cases=77, controls=931) in GS:SFHS is shown in **Table 5.2**.

	TR	Neuroticism	Resilience	Task-orientated coping	Emotion-orientated coping	Avoidance-orientated coping
TR	-					
Neuroticism	0.15 $P=3.5x10^{-6}$	-				
Resilience	-0.13 $P=2.8x10^{-5}$	-0.38 $P=2.2x10^{-16}$	-			
Task-orientated coping	-0.08 $P=0.010$	-0.21 $P=1.4x10^{-11}$	0.37 $P=<2.2x10^{-16}$	-		
Emotion-orientated coping	0.06 $P=0.042$	0.41 $P=<2.2x10^{-16}$	-0.53 $P=<2.2x10^{-16}$	-0.31 $P=<2.2x10^{-16}$	-	
Avoidance-orientated coping	-0.12 $P=0.00014$	-0.037 $P= 0.037$	0.11 $P=0.00068$	0.35 $P=<2.2x10^{-16}$	0.11 $P=0.00081$	-

5.2. Pearson's correlation matrix of treatment resistance, neuroticism, resilience and coping styles. Abbreviation: TR, treatment resistance.

Standardised beta coefficients from the logistic and probit (structural equation modelling mediation) regression models are reported throughout for consistency and comparison between models. A positive beta coefficient indicates a greater association with TR and can be interpreted as the probability that TR=1 conditioned on the covariates in the model.

Independent associations

All univariate associations demonstrated significant association with TR; neuroticism (P_{FDR}= 6.0x10^{-9}, beta= 0.47, R^2= 0.20), BRS (P_{FDR}= 6.0x10^{-8}, beta= -0.66, R^2= 0.049), task-orientated (P_{FDR}= 0.018, beta= -0.020, R^2= 0.0089), emotion-orientated (P_{FDR}= 0.00016, beta= 0.036, R^2= 0.025) and avoidance-orientated coping (P_{FDR}= 0.00027, beta= -0.041, R^2= 0.021; **Table 5.3**).

	P_{FDR}	Beta	R^2	N (cases)
Neuroticism	**6.0x10^{-9}**	**0.47**	**0.20**	**2390 (262)**
BRS	**6.0x10^{-8}**	**-0.66**	**0.049**	**1255 (96)**
Task-orientated coping	**0.018**	**-0.020**	**0.0089**	**1176 (89)**
Emotion-orientated coping	**0.00016**	**0.036**	**0.025**	**1216 (95)**
Avoidance-orientated coping	**0.00027**	**-0.041**	**0.021**	**1208 (98)**

Table 5.3. Independent associations of stressful life events, coping styles and resilience on antidepressant resistance. Significant values (P_{FDR}<0.05) are shown in bold. Abbreviations: BRS, Brief Resilience Scale; FDR, false discovery rate.

Moderation

No evidence of moderation was identified in any model. After fitting both neuroticism and resilience, BRS and not neuroticism was significantly associated with TR. Neuroticism was significantly associated with TR after correcting for the three coping styles whereas only avoidance-orientated coping style was significantly associated with TR after accounting for neuroticism (**Table 5.4**).

	Variable 1		Variable 2		Interaction		
	P_{FDR}	Beta	P_{FDR}	Beta	P_{FDR}	Beta	N (cases)
Neuroticism and BRS	0.075	0.38	**0.0078**	**-0.44**	0.98	-0.0087	1072 (65)
Neuroticism and Task-orientated coping	**0.0037**	**0.45**	0.18	-0.17	0.98	0.0025	1015 (76)
Neuroticism and Emotion-orientated coping	**0.0037**	**0.44**	0.18	0.018	0.98	-0.0061	1045 (65)
Neuroticism and Avoidance-orientated coping	**0.0028**	**0.45**	**0.0078**	**-0.041**	0.98	-0.0036	1036 (82)

5.4. Moderation results of neuroticism, resilience and coping styles on treatment resistance. Significant values (P_{FDR}<0.05) are shown in bold. Abbreviations: BRS, Brief Resilience Scale; FDR, false discovery rate.

Full model

Full results are detailed in **Figure 5.5.**

Model with task-orientated coping

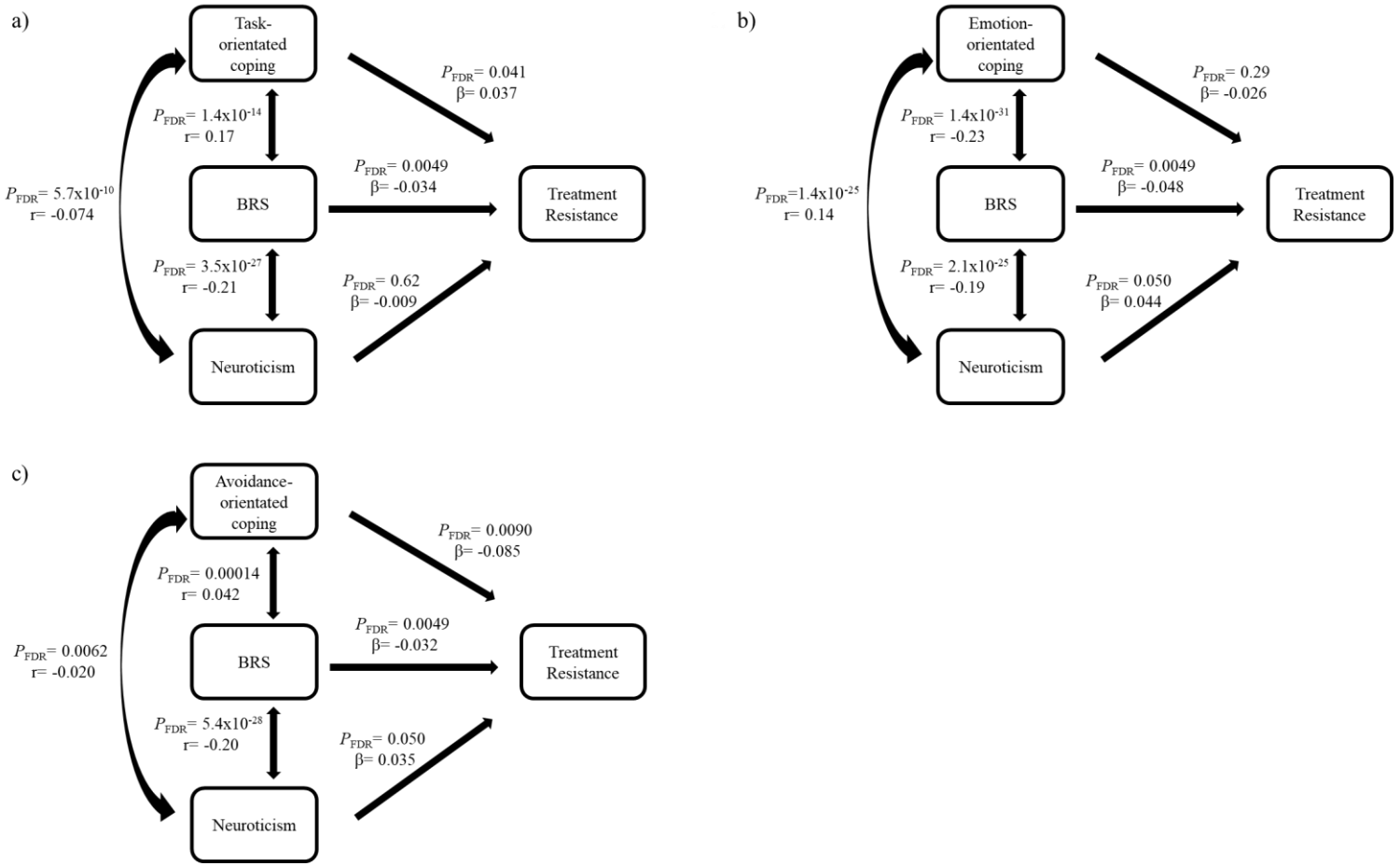
The model included the effects of task-orientated coping, BRS and neuroticism on TR whilst also reporting each correlation coefficient between the independent variables. The fit statistics for this model indicated a good model fit (CFI= 0.99, TLI= 0.98, RMSEA= 0.030).

Model with emotion-orientated coping

The model included the effects of emotion-orientated coping, BRS and neuroticism on TR whilst also reporting each correlation coefficient between the independent variables. The fit statistics for this model indicated a good model fit (CFI= 0.98, TLI= 0.98, RMSEA= 0.037).

Model with avoidance-orientated coping

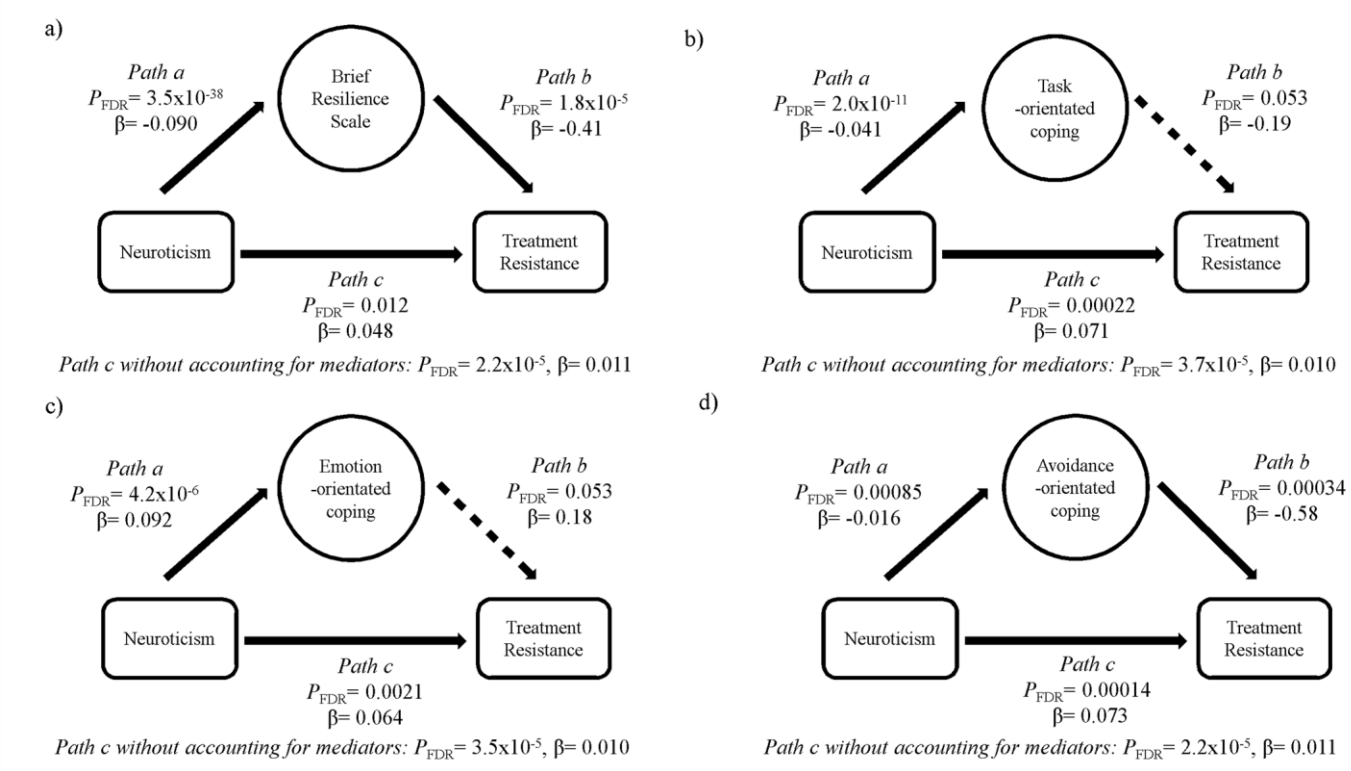
The model included the effects of avoidance-orientated coping, BRS and neuroticism on TR whilst also reporting each correlation coefficient between the independent variables. The fit statistics for this model indicated a poor model fit (CFI= 0.85, TLI= 0.84 0.98, RMSEA= 0.072).



5.5. Full models for neuroticism, resilience and coping style associated with treatment resistance. Three different models to demonstrate the effect of a full model with (a) task-orientated coping, (b) emotion-orientated coping and (c) avoidance orientated coping. Standardised betas and correlations between variables are reported.

Mediation

For each mediation model, a significant indirect association (paths a and b) represents evidence of mediation and both a significant indirect and direct association (path c) represents evidence of partial mediation. For the individual path effect sizes (a, b and c), see **Figure 5.6**.



5.6. Mediation models for neuroticism and treatment resistance mediated by resilience and coping style. In each model, the direct association is between neuroticism and TR with the mediators being (a) resilience, (b) task-orientated coping, (c) emotion-orientated coping and (d) avoidance orientated coping. Significant pathways are represented by a solid arrow and non-significant paths are represented by a dashed line. Path c is shown with and without accounting for the effects of mediation. Abbreviations: FDR, false discovery rate.

Full tabulated results for mediation models are given in **Supplemental Table 2** and all fit statistics are given in **Supplementary Table S3**. All factor loadings for the latent variables are given in **Supplementary Tables S4-7**.

BRS as the mediator

A direct path between neuroticism and TR (path c) was found when including the mediating effect of BRS and accounted for 4.8% of the variance. The indirect path (path ab) was also significant (P_{FDR}= 3.4x10^{-5}) and indicated that lower BRS partially mediates 43% of the association between neuroticism and TR. The fit statistics for this model indicated a good model fit (CFI= 0.97, TLI= 0.96, RMSEA= 0.048).

Task-orientated coping as the mediator

A direct path between neuroticism and TR (path c) was found when including the mediating effect of task-orientated coping and accounted for 7.1% of the variance. The indirect path (path ab) was not significant (P_{FDR}= 0.062) and indicated that task-orientated coping did not mediate the association between neuroticism and TR. The fit statistics for this model indicated a good model fit (CFI= 1.0, TLI= 1.0, RMSEA= 0.013).

Emotion-orientated coping as the mediator

A direct path between neuroticism and TR (path c) was found when including the mediating effect of emotion-orientated coping and accounted for 6.4% of the variance. The indirect path (path ab) was not significant (P_{FDR}= 0.062) and indicated that emotion-orientated coping did not mediate the association between neuroticism and TR. The fit statistics for this model indicated a good model fit (CFI= 0.98, TLI= 0.97, RMSEA= 0.036).

Avoidance-orientated coping as the mediator

A direct path between neuroticism and TR (path c) was found when including the mediating effect of avoidance-orientated coping and accounted for 7.3% of the variance. The indirect path (path ab) was significant (P_{FDR}= 0.016) and indicated that lower avoidance-orientated coping partially mediates 11% of the association between

neuroticism and TR. However, the fit statistics for this model indicated a poor model fit (CFI= 0.81, TLI= 0.79, RMSEA= 0.071).

5.2.5. Discussion

To our knowledge, this is the first study to examine the mediating and moderating effects of resilience and coping style of neuroticism's association with antidepressant treatment resistance. An initial independent association analysis demonstrated that all variables were significantly associated with TR. Neuroticism was positively associated with TR and explained 20% of the variance, replicating previous findings (Bock et al., 2010; Quilty, De Fruyt, et al., 2008). Our results indicated that lower resilience was associated with a higher probability of a TR outcome and explained 5% of the variance. Furthermore, both task-orientated and avoidance-orientated coping were negatively associated with TR (explaining 1% and 2% of the variance, respectively) whilst emotion-orientated coping was positively associated with TR (explaining 3% of the variation).

Resilience has not been previously associated with TR using a quantitative measure, although previous studies have demonstrated that higher resilience (measured by the resilience scale for adolescents) is associated with fewer depressive symptoms (Hjemdal, Aune, Reinfjell, Stiles, & Friborg, 2007). Similar directions of effect have been reported in MDD with task-orientated and emotion-orientated coping (McWilliams, Cox, & Enns, 2003), but inconsistent results have been reported for avoidance-orientated coping. Whilst some studies have reported avoidance-orientated coping as a risk factor for depression and depressive symptoms (Silverstein et al., 2016; Tan-Kristanto & Kiropoulos, 2015), others have demonstrated an association with positive emotions in the short-term but negative outcome in the long term (M. M. Smith, Saklofske, Keefer, & Tremblay, 2016). In this study, we demonstrate that higher avoidance-orientated coping was associated with antidepressant responders. The impact of coping style on TR has never been previously reported.

In the full models of the variables, neuroticism was no longer significantly associated with TR after accounting for task-orientated coping and resilience whereas emotion-orientated coping was not associated with TR after accounting for resilience and neuroticism. Avoidance orientated coping, neuroticism and resilience were all significantly associated with TR but this model was not a good fit to the data. These results indicate that the variables were not independently associated when accounting

for each other which could be explained by correlation between the variables or interaction. Given that coping style and neuroticism were weakly correlated and coping style and resilience modestly correlated, this result indicates that there could be interaction between the predictors.

We found no significant moderation of neuroticism on TR by coping styles or resilience. Mediation analysis indicated that resilience partially mediated the association between neuroticism and TR. Specifically, resilience was found to mediate 43% of the association. However, as this mediation was only partial, neuroticism remained independently directly associated with TR. We demonstrate that low neuroticism is associated with an increased likelihood of resilience and a decreased likelihood of a TR outcome. Neither task-orientated or emotion-orientated coping mediated the effect of neuroticism on TR but was associated with neuroticism itself in both instances. This therefore suggests that neuroticism was accounting for the effect of task-orientated and emotion-orientated coping on TR. Additionally, whilst mediating effects of avoidance-orientated coping were found, the model's poor fit statistics mean these results should be discounted. It is perhaps surprising, considering previous research demonstrating associations with maladaptive coping and neuroticism in over 4,600 individuals (Afshar et al., 2015), emotion-orientated and avoidance-orientated coping were not mediators.

In this study, we assume that neuroticism is stable over the lifetime but it has been shown that neuroticism is liable to changes with changes depression (Chow & BW, 2014) and stressful life events can modestly increase neuroticism (Riese et al., 2013). Moreover, changes in neuroticism trait levels can occur whilst on SSRI treatment (Quilty, Meusel, et al., 2008). Nonetheless, Renner *et al.,* (2013) found that neuroticism score is not likely altered after undergoing treatment with various antidepressants (Renner, Penninx, Peeters, Cuijpers, & Huibers, 2013). Considering these inconsistent results, additional studies examining neuroticism changes over the course of antidepressant treatment may be beneficial. It is possible that treatment resistant individuals have a higher depression severity which is more difficult to treat with traditional antidepressants. In fact, neuroticism has been shown to be associated with higher depression symptom severity and the relationship was mediated by

maladaptive coping styles (Yoon et al., 2013). However, whilst some studies have indicated antidepressants are more successful in a clinically severe population rather than mild or modest depression (Fournier et al., 2010) others have reported no difference between severity groups (Rabinowitz et al., 2016).

One of the most widely reported theories for the mechanism by which neuroticism increases risk for depression is through increasing an individual's liability to stress (Klein, Kotov, & Bufferd, 2011) and chronic stress has been shown to moderate its effect on the course of depressive symptoms (Brown & Rosellini, 2011). Stress is involved in several hypothesized biological mechanisms of depression including the brain-derived neurotrophic factor (BDNF) hypothesis, the cytokine hypothesis and hippocampal neurogenesis (Stepan et al., 2015; Warner-Schmidt & Duman, 2006) which have all been implicated in antidepressant response (Björkholm & Monteggia, 2016; Eliwa et al., 2017; Uher et al., 2014). In fact, it has been shown that antidepressants are only able to exert an effect if hippocampal neurogenesis mechanisms are intact (Surget et al., 2011). As resilience and coping styles effect response to stress, their role in the neuroticism could also be related to stressful life events. Nevertheless, neuroticism was only partially mediated by resilience meaning that this could only explain the association in part. Further research into neuroticism's relationship with stress and its biological mechanisms in TR would be beneficial.

We replicate the finding that neuroticism is important in the prediction of TR and further add that resilience partially mediates this mechanism. It has been previously demonstrated that both task-orientated and emotion-orientated coping are significant contributors to resilience (Campbell-Sills et al., 2006) and therefore, resilience is likely a construct that incorporates coping style. Although replication and further research is necessary before making any clinical recommendations, the results of this study indicate that modifying coping style could facilitate response to antidepressants. Coping styles have been previously reported as modifiable (Cairns, Yap, Pilkington, & Jorm, 2014).

Certain limitations in this study deserve consideration. Firstly, it should be highlighted that the sample size in this analysis was small (largest sample for moderation/mediation analysis: 954 non-TR controls and 82 TR cases), therefore,

further analysis utilising larger sample sizes may be beneficial especially in generating more accurate effect sizes. Prescription records in GS:SFHS were only available for a six-year period meaning that antidepressant prescriptions prior to 2009 were not captured. Furthermore, although exclusions on dose were carried out using BNF recommendations for MDD and schizophrenia, schizoaffective and bipolar individuals removed, it is possible that individuals could have been prescribed antidepressants for other indications including anxiety disorders, post-traumatic stress disorder, obsessive compulsive disorders, panic disorders and non-psychiatric indications. It is also possible that drug switching occurred due to adverse effects, however, this is thought to occur prior to six weeks which was the minimum duration in this study. Finally, neuroticism, TR and coping styles were all measured at different time points; neuroticism was measured between 2006-2011, TR was defined between 2009-2015 and coping styles were measured between 2014-2017. Although TR would have been defined subsequent to measures of neuroticism for most individuals, it was defined retrospectively to coping styles. Evidence has shown that coping styles can be marginally modified (Nielsen & Knardahl, 2014), specifically, maladaptive coping styles (emotion and avoidance coping styles) have been shown to be more susceptible to change in comparison to adaptive coping styles (task-orientated coping) (Franken, Hendriks, Haffmans, & van der Meer, 2001). Relatively few studies have explored the effect of antidepressant treatment on coping style itself. A small study of 16 dysthymic patients, 17 MDD patients and 18 controls reported that depression recovery after SSRI treatment is associated with a decreased reliance on emotion-orientated coping (Ravindran, Griffiths, Waddell, & Anisman, 1995). Additionally, vortioxetine has been shown to restore adaptive coping in stressed rats (Hatherall, Sánchez, & Morilak, 2017). Nevertheless, no large study exploring the effect of antidepressants on coping style longitudinally has been carried out. Therefore, careful interpretation should be applied and change in coping style during antidepressant treatment should be further examined.

In conclusion, we demonstrate that neuroticism, resilience and coping styles are each independently associated with a TR outcome (as shown in the univariate analysis), presenting potentially modifiable risk factors. Furthermore, we demonstrate that resilience partially mediates the relationship between neuroticism and TR.

Acknowledgements

We thank all families, practitioners and the Scottish School of Primary Care involved in the recruitment process as well as the entirety of Generation Scotland team; interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, healthcare assistants and nurses. We are grateful to the Sackler Foundation for the generous support of this work. IJD is supported by MRC and BBSRC funding to the University of Edinburgh Centre for Cognitive Ageing and Cognitive Epidemiology (MR/K026992/1).

Financial support

This investigation was supported by the Wellcome Trust 104036/Z/14/Z (STRADL, Stratifying Resilience and Depression Longitudinally). Generation Scotland received core funding from the Chief Scientist Office of the Scottish Government Health Directorate CZD/16/6 and the Scottish Funding Council HR03006.

Conflict of interest

AMM has received financial support from Pfizer (formerly Wyeth), Janssen and Lilly. The remaining authors declare no conflict of interest.

5.3. Chapter Conclusion

This study represents the largest analysis of neuroticism in antidepressant resistance to date and replicates previous findings of positive associations between neuroticism and TR whilst also demonstrating an additional a role of coping styles and resilience on TR. No coping styles (task-orientated, emotion-orientated or avoidance-orientated coping) mediated the relationship between neuroticism and TR but lower resilience partially mediated the effects of higher neuroticism. Partial mediation between of stress on the association between neuroticism and depressive symptoms has previously been reported (S. E. Kim et al., 2016). This is indicative that although neuroticism influences MDD and antidepressant resistance through stress-related risk factors, there may be other biological components involved. Kim *et al.,* (2016) also demonstrated that females show higher levels of neuroticism which contributed to more depressive symptoms compared to men (S. E. Kim et al., 2016). Gender differences were not explored here due to modest sample size; however, future studies would benefit from sample size enhancements and further explication of potential gender effects.

Neuroticism has been negatively associated with the placebo effect as individuals with higher neuroticism are more inclined towards negative attitudes to pharmacological treatments (Jakšić, Aukst-Margetić, & Jakovljević, 2013). Previous research has shown higher placebo effect to be associated with mild-moderate responders (Kirsch et al., 2008). Clinical trials reduce the likelihood of placebo responders by selecting more severe depression cases and by randomising people blindly to treatments. In this present study, depression severity was not accounted for and antidepressant users in the general population were examined, therefore it is likely that there are placebo responders within this sample. The association between neuroticism and treatment resistant therefore could be partially attributed to this.

Neuroticism demonstrates a significant predictor of antidepressant treatment resistance, explaining 20% of the variance in TR. However, the mechanism for this association is still unexplained. Future studies would benefit from larger samples to explore the effect of stress-related traits as well as the role of the placebo effect.

Chapter 6. Discussion.

6.1. Summary

This thesis presents three studies that seek to investigate intermediate phenotypes in MDD with a view to reducing the causal heterogeneity associated with the clinical phenotype. Given the previous literature documenting the high heterogeneity and modest heritability of MDD, genetic stratification illustrates a potentially effective technique to divide MDD into aetiologically-defined groups (Chapter 2). This thesis had two main objectives; (1) explicating the genetic overlap between regional brain volumes and MDD, and (2) enhancing current sample sizes in pharmacogenetic studies to identify potential mechanisms underlying MDD stratified by treatment response. In Chapter 3, the intermediate phenotype approach was adopted whereby regional brain volumes and MDD were examined for both overlap and subgrouping. In Chapters 4 and 5, antidepressant treatment resistance was examined using genetic and non-genetic approaches in order to explicate pathways to non-responsiveness. Here, I will briefly summarise the results and main findings.

Regional brain volume and MDD

Previous work researching subcortical volumes and MDD primarily explored the effects of candidate genes (which are prone to selection bias). Therefore, the genome-wide approach applied in this thesis provides a hypothesis-free approach that is less susceptible to selection bias, and for which there are established methods for controlling for population stratification and false positives. Sample sizes were adequate to reject a modest to high genetic correlation between all volumes (excepting hippocampal volume) and MDD therefore not supporting previous findings of a potential genetic overlap, although this work does not exclude the possibility of a low to modest genetic correlation. Nevertheless, the general literature supports an association between brain volume and MDD. It is therefore possible, if not likely, that there is an environmental component that affects brain volume in MDD patients. In fact previous studies have found that the effect of early life adversity on MDD was partially mediated by smaller hippocampal volume (Rao et al., 2010). Gene-

environment interaction could also explain the phenotypic associations and previous research has indicated its role in brain volumes in MDD patients, however this research has also primarily focused on candidate genes (Frodl et al., 2010; Verhagen et al., 2010).

The positive genetic correlation between hippocampal volume and MDD reported in this chapter was not significant after correcting for multiple testing. Given this and that it is in the opposite direction to previously reported associations, it is possible that this finding represents type I error. An alternate explanation to this is that, as indicated in the BUHMBOX analysis, the positive correlation is present in a subgroup of MDD patients, nevertheless, this did not replicate in two other independent cohorts. Therefore, further analysis is needed in order to evaluate the role of hippocampal volume in MDD.

In summary, the lack of strong genetic correlation reported in Chapter 3 does not support the function of regional brain volumes as intermediate phenotypes for MDD. Nevertheless, environmental influences, such as stressful life events, may still be important in the role of regional brain volumes in MDD pathology and genetic correlation may still be present at levels lower than we had power to detect.

Factors involved in antidepressant treatment resistance

The largest current meta-analysis in antidepressant non-response (in a clinical sample) is comprised of ~2,900 individuals (Tansey et al., 2012). In Chapter 4, antidepressant resistance was defined using prescription records in a population-based cohort and meta-analysed with GENDEP with the aim of surpassing this number and allowing the application of techniques for exploring genetic overlap and intermediate phenotypes. No variants, genes, pathways or PRS associated with treatment resistance or stages of resistance, but PRS analysis was only adequately powered to determine an association between schizophrenia PRS and stages of resistance. However, this study was in part a proof-of-concept study demonstrating the use of prescription records in antidepressant analysis. Other biobanks with access to prescription records may have sufficient numbers for a well-powered genome-wide association study in treatment resistance.

Pedigree-based genetic correlations were found between neuroticism, psychological distress, mood disorder personality and schizotypal personality (in stages of resistance only) indicating that exploring them as potential intermediate phenotypes may be beneficial. Similarly, neuroticism, coping styles and resilience were all associated with treatment resistance outcome in Chapter 5. To confirm intermediate phenotype status, these would have to be explored in further detail. The genetic and phenotypic associations here could also indicate the presence of pleiotropy. Pleiotropy can be biological (a gene or variant having an influence on multiple traits), mediated (a gene or variant influencing one trait in order to affect another) or spurious (false association). A causal pathway from gene through intermediate phenotype would have to be established. Should these phenotypes demonstrate to be intermediaries, several suggestions could explain their relationship. It is possible that the intermediate phenotypes could represent a subgroup of individuals and further explication could identify aetiological subgroups, whilst is also possible that they could represents misclassification, whereby those with high mood disorder personality could be misclassified bipolar cases. Further explication of the mechanisms driving the genetic correlation would be necessary to provide an explanation for the relationships.

In summary, these studies demonstrate strong support for the association of neuroticism with antidepressant resistance with it being both phenotypically associated (explaining 20% of the variance) and genetically correlated (r_g= 0.66 and r_g= 0.51 with TR and SR, respectively). The partial mediation of resilience indicates that although stress-associated factors may have a part role in neuroticism's association with TR they do not fully explain it.

6.2. Limitations

The majority of limitations have been addressed in each individual chapter, therefore here, I will only comment on the main limitations. Additionally, an overview of the limitations related to the definition of antidepressant resistance will be explored.

The major contributing limitation to almost every analysis in this thesis was insufficient power, despite each chapter providing some of the largest reported sample

sizes. In Chapter 3, power was insufficient to detect a genetic correlation of less than 0.24 for the putamen and 0.49 for the nucleus accumbens. Therefore, to adequately assess the presence of genetic correlations below these values, it would be necessary to replicate utilising data from larger samples. In Chapter 4, to detect an allele with a MAF of 5% and an OR of 1.6, analysis of TR would need 1,624 cases whereas we reported effects with 358 cases. Furthermore, in Chapter 5, the sample size for moderation and mediation only consisted of a maximum of 82 cases. Nevertheless, these were the largest available samples at the time of analysis therefore future investigations would benefit from significantly enhancing sample sizes in clinical trials, or drawing together larger samples through meta- and mega-analysis.

The role of the placebo effect is a common complication within antidepressant response studies as identification of placebo responders is exceedingly difficult. Considering it was not possible to select individuals based on depression severity in GS:SFHS (a commonly used approach to reduce placebo responders in clinical trials), it is likely that placebo responders are present in our sample. As a result, this makes the association with neuroticism potentially more difficult to interpret. Individuals with higher neuroticism scores are more prone to pharmacophobia (Jakšić et al., 2013) and, consequently, neuroticism may be negatively associated with treatment resistance due to lower neuroticism scores in treatment responders driven by the placebo effect. Placebo responders have been shown to account for 35-40% of antidepressant response (Furukawa et al., 2016), but their occurrence is still widely debated (Bschor & Kilarski, 2016).

Neuroticism was explored as a stable trait throughout this thesis, however, neuroticism is subject to changes in certain disease states. Specifically, neuroticism score has been shown to increase after a stressful life event (Riese et al., 2013). Additionally, changes in neuroticism have been shown to occur with changes in depression (Chow & BW, 2014) whilst changes in neuroticism on antidepressants has also been shown to be subject to change (Quilty, Meusel, et al., 2008). It may therefore be more valuable to measure neuroticism longitudinally with depression to assess trait change associated with illness.

Finally, employing the intermediate phenotypes approach is not without its caveats. The involvement of epistasis and gene-environment interactions are not accounted for utilising these approaches but are both likely to be involved in the genetic architecture of MDD (Lopizzo et al., 2015; Schott et al., 2014). Moreover, these techniques are reliant on GWAS data which require large sample sizes to be representative of the genetic architecture of a trait. It is necessary then that adequately sized samples are available for each examined trait which is often not the case. GWAS also only examines common variants that confer relatively small effects in comparison to rare variants. The most accurate approach to a genetic study would be one that incorporates common variants, rare variants, epistasis and gene-environment interactions. However, no approach to date has yet been able to achieve this task. Consequently, intermediate phenotypes remain an effective approach to analyse the current data and identify potential clinical subgroups.

Defining antidepressant resistance in a population-based cohort

Electronic health records can contain a multitude of information including varying levels of details from prescription records. Nevertheless, prescription records often do not contain response data meaning the approach of inferring response after adequate treatment, whilst practical and necessary, may have limitations. An adequate antidepressant treatment is usually defined as one that reaches a minimum dose threshold and minimum duration, which are defined by clinical guidelines in each country. Clinical prescription guidelines in the UK are detailed in the British National Formulary (BNF). For antidepressants, the BNF currently recommends a minimum duration of four weeks (Joint Formulary Committee, 2017) with studies showing that response can occur between four and six weeks (Hansen, Gartlehner, Lohr, Gaynes, & Carey, 2005). Consequently, a maximum of six weeks on an antidepressant is considered to be more than satisfactory to elicit response and has been labelled the 'turning point' whereby additional treatment options such as administering a higher dose or different antidepressant are considered (Blier, 2009). Nevertheless, it has historically been shown that, due to interindividual variability in response, longer treatments and higher doses in non-response may be effective in some patients (Targum, 2014). In this thesis, BNF recommendations for daily dose were used to

ensure the minimum dose required for clinical effect. A six-week threshold was also adopted in order to apply a more conservative approach whilst also accounting for any individuals that may have switched drugs due to side effects. Nonetheless, it is possible that these exclusion thresholds removed those that would have been eligible for inclusion, if more detailed phenotyping information had been available. Furthermore, no information on drug response was available in the prescription records, therefore, drug switching was used to infer failure to a drug. This method has been applied before in treatment resistant schizophrenia and in treatment resistant depression (O'Dushlaine et al., 2014; Ruderfer et al., 2016; Wimberley et al., 2016), but is prone to error. For instance, it is possible that the individuals switched drugs due to side effects or never completed their antidepressant prescription, low adherence to antidepressants is commonly reported (Keyloun et al., 2017; Leggett, Ganoczy, Zivin, & Valenstein, 2016). An additional limitation to this method is that it does not account for combination therapies. Although in the UK it is generally considered that combination therapies are trialled after failure of a second single antidepressant trial (Joint Formulary Committee, 2017), at which point, by the definition in thesis, they would already be considered treatment resistant.

In antidepressant response, there is disparity between studies defining treatment resistance. Non-response after one and two trials have been proposed for TR (Souery et al., 1999). In 2006, the STAR*D trial described an inflection point in response after two treatments (A. J. Rush et al., 2006), consequently, general consensus is that it should be described as non-response to more than two adequate treatment trials (Conway et al., 2016). However, it has been additionally proposed that a staging model of antidepressant response more accurately describes resistance whereby stages are given based on number of antidepressant trials, class of antidepressant, dose and duration. Several different staging models have been suggested (Conway et al., 2016; McIntyre et al., 2014) but these have received criticism for amalgamating multiple classes and increasing heterogeneity in the trait (Fogelson & Leuchter, 2017). Nevertheless, no general consensus has been reached on the effectiveness of between-class switching as switching between different antidepressant classes has not generally shown different efficacy than switching within the same class (Souery et al., 2011).

6.3. Future Work

Although the work in this thesis does not generally support the role of subcortical volumes as genetic intermediate phenotypes for MDD, meta-analyses have generally supported a phenotypic association, albeit results have been somewhat inconsistent (Schmaal et al., 2015; Sexton, Mackay, & Ebmeier, 2013). Relative contributions of genetic and environmental factors differ per brain region, but in general brain volumes have been reported to be associated with both shared and independent environments (Blokland, de Zubicaray, McMahon, & Wright, 2012) therefore additional exploration into an environmental effect to may explain the association. Furthermore, given the ascertainment differences between the samples applied to BUHMBOX in Chapter 3, it would be advantageous to replicate the study in a clinically defined sample to determine the presence of a true subgroup. Moreover, the ENIGMA consortium is currently conducting a large GWAS meta-analysis of cortical volumes, therefore similar genome-wide approaches could be applied to cortical structures.

The major obstacle to pharmacogenomic investigation of antidepressant treatment resistance is sample size. In this thesis, an approach to circumvent small sample sizes in pharmacogenomic research is demonstrated whereby antidepressant treatment resistance is defined utilising prescription records. This is arguably a better approach than self-report methods as these methods rely on retrospective accounts of individuals which are typically less reliable. Access to electronic health records is becoming increasingly available in large national biobanks. The first recorded national biobank was formed in Iceland but since its outset, several nation-wide cohorts have been established; Singapore, Sweden, Denmark, Japan and others (Mitchell, 2010). In the UK for instance, UK Biobank has genetic data collected for 500,000 individuals with access to medical records becoming available to researchers with the next few months. Prior to the ENIGMA project, neuroimaging research was prone to similar complications as pharmacogenomic research. By utilising meta-analysis and enhancing sample sizes in biobanks, research in neuroimaging is now reaching an appropriate number for genome-wide investigations. In antidepressant pharmacogenomics, a large collaborative meta-analysis (such as ENIGMA), utilising the cohorts already available along with data from the national biobanks, may

demonstrate an effective method to increase numbers to allow genome-wide analysis to be possible.

Subsequent to these sample increases, multiple further approaches could be applied to explicate antidepressant resistance. Firstly, it would be beneficial to generate an empirical definition of treatment resistance and/or a staging model so it may be applied to future research. Secondly, it may be beneficial to examine regional brain volumes as an intermediate phenotype to antidepressant resistance (using similar approaches as Chapter 3) as regional brain volumes have been previously associated with antidepressant non-response (Fu et al., 2013). Thirdly, genomic information (such as PRS) could be assessed within mediation models in order to examine the pathways from genes to outcome. Fourth, exploration into potential gender differences in antidepressant response is warranted as previous studies have shown that women preferentially respond to SSRIs whereas men typically respond better to tricyclic antidepressants (Kornstein et al., 2000). Lastly, change in neuroticism and coping style during antidepressant treatment should also be investigated as neuroticism change has been shown to be mediate SSRI response (Quilty, Meusel, et al., 2008). Furthermore, resilience only partially explained the mechanism by which neuroticism induced antidepressant non-response indicating alternative pathways should be explored.

In both MDD and antidepressant resistance the effects of rare variants should be examined, once adequate numbers have been reached. The role of rare variants may be of value as they typically confer larger effects in a subpopulation of individuals. Consequently, they provide more effective targets for pharmaceutical intervention with their biological consequences eluding more about the pathology of the disease. Moreover, rare variant analysis may aid in genetic stratification given that these variants are only present in population subgroups.

Epigenetics and their role in MDD and antidepressant resistance also provide future potential. Epigenetics is the occurrence of processes such as methylation which can turn a gene “on” or “off”. It is instigated by an external modification, such as a stressful event, that leads to change in the function/structure of DNA. Numerous studies have demonstrated that early adversity has subsequent epigenetic effects especially amongst genes involved in the HPA axis, serotonin transporter and BDNF

(Jawahar, Murgatroyd, Harrison, & Baune, 2015; Menke & Binder, 2014). Furthermore, epigenetics mechanisms are known to play a role in antidepressant response. Antidepressant non-responders have exhibited lower BDNF messenger ribonucleic acid (mRNA) expression levels than responders and treatment with a SSRI caused an increase in methylation with methylation differences predicting drug response (Menke & Binder, 2014). Future analysis may benefit from a better understanding of the epigenetic factors involved in MDD and antidepressant resistance.

To confirm intermediate phenotype status, further research would be required. Intermediate phenotypes need to meet several criteria, including being associated with the disease of interest and being significantly heritable. There are two models of intermediate phenotype status, the mediation model and the liability-index model. In order to test the mediation model it would be necessary to demonstrate a causal association with the disease. Analysis such as mendelian randomisation could be utilised to determine this. For the liability-index model the two phenotypes do not need to be associated causally and a pleiotropic relationship could explain their relationship, by indicating that a subset of genes cause both traits. Although significant genetic correlation indicates a relationship, further exploration into which model best explains the relationship is needed. To further explore the intermediate phenotype status of neuroticism and which model best explores the relationship, further analysis with techniques such as mendelian randomisation would be necessary.

This thesis has focused on the intermediate phenotype approach to genetic stratification, but it may also be valuable to apply machine learning approaches. Regular advancements in these techniques are likely to equip them to be invaluable tools for prospective use. Machine learning methodologies are becoming increasingly popular in cancer research and are infrequently being applied to psychiatric research. Genetic stratification using both these methodologies could significantly benefit both MDD and antidepressant treatment resistance.

6.4. Potential clinical applications

MDD is a highly complex and heterogeneous disorder that is attributed to multiple genetic and environmental components, and this thesis demonstrates only some. Individually each component contributes only a little of the total variance of MDD but together this could additively explain much more. It has therefore been proposed that prediction models could be used to identify patients at high risk of developing MDD. For instance, MDD prediction models in children have demonstrated area-under-curves (AUC) of between 0.70 and 0.72 using social factors, comorbidities, symptoms and drug and alcohol misuse (Nichols, Ryan, Connor, Birchwood, & Marshall, 2016). Similar models have been constructed in treatment resistant depression, the most recent of which reported a prediction accuracy of 0.74 for treatment resistance and 0.85 for treatment remission (Kautzky et al., 2017). The addition of genetics in these models may also increase accuracy especially with further understanding of gene-environment interactions. By identifying high risk individuals, early intervention strategies could be implemented to target the individual prior to the onset of the illness. Similarly, by identifying those that are less likely to respond to antidepressants, alternative treatment methods can be implemented. A similar prediction model of response to psychotherapies may also be beneficial.

Given larger sample sizes, GWAS analysis in treatment resistant depression may have power to detect genetic associations. This could reveal mechanisms to non-response which may define separate aetiology. With this information, it may be possible to construct pharmacotherapies that target the underlying mechanism of disease. Common genetics are often criticised for low effect sizes, however, the effect of statins on cholesterol is 20 times stronger than the effect reported for HMCGR (the statin receptor) genetic variants from GWAS analysis (Barrett, Dunham, & Birney, 2015). This highlights the importance of these kinds of studies for elucidating potential mechanisms and producing more effective and targeted treatments.

6.5. General Conclusion

Genetic stratification of MDD may aid in identifying more aetiologically-defined subgroups that can be better targeted by treatments. This thesis demonstrates

application of the intermediate approach with two objectives: assessing the genetic overlap between regional brain volumes and MDD and exploring antidepressant treatment resistance. No genetic overlap between regional brain volume and MDD was identified and therefore these results do not support the role of subcortical brain volumes as intermediate phenotypes. However, neuroticism was indicated as an intermediate phenotype in antidepressant resistance. Furthermore, its association was partially mediated by resilience. This work in this thesis highlights the importance of large sample sizes. The most important next steps for explicating antidepressant resistance will be enhancing current sample sizes and advocates the initiation of an international consortium to meta-analyse current data and recruit larger biobanks. Only after this, will it be possible to explicate the biological mechanisms that underlie non-response and use this information to stratify MDD. Explication and application of genetic stratification using the techniques described here may aid in reducing the heterogeneity of MDD and assist in the generation of more targeted treatments.

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Appendices

Appendix 1. Supplementary Material to Section 3.2.

Supplementary material for the manuscript by Wigmore et al "Shared Genetic Architecture of Regional Brain Volumes and Major Depressive Disorder in Generation Scotland (n=19,762), UK Biobank (n=24,048) and the English Longitudinal Study of Ageing (n=5766)".

Contents:

Supplemental Methods

Cohort descriptions and MDD phenotype

GS:SFHS: GS:SFHS is a cohort of 24,080 participants collected from Scottish medical practices between 2006 and 2011. Participants were recruited at random and were eligible if they were over 18 years of age and had one first-degree relative also willing to participate. Genotype data was available for 20,032 participants which after QC was reduced to 19,994 (males=8,158, females=11,698). 2,643 participants (13.4%) were given a diagnosis of MDD after SCID interview with 17,119 controls. Episode data was available for 2,643 cases and was coded from 0 to 40 episodes with anyone above that value (including those who had more episodes than they could recall) given the value 41. Age of onset information was available for 2,631 cases. For recurrent MDD, single episode MDD were excluded leaving 1,319 cases. MDD duration was calculated by age of onset subtracted from age of participant.

UK Biobank: UK Biobank is an open access cohort of 502,664 individuals collected from across the United Kingdom between 2006 and 2010. Participants were eligible if they were between the age of 40 and 69. After QC, genotype data was available for 152,734. Related individuals and GS and ELSA participants were excluded from this sample leaving 116,909 individuals (males=55,410, females=61,499). 8,146 MDD cases (33.9%) were available in this sample and 15,886 controls, a large number of controls were excluded due incomplete or missing data explaining the higher proportion of cases. MDD episode data was available for 6,466 individuals and coded as for GS:SFHS, therefore, recurrent MDD (excluding any single episode MDD) included 4,612 cases. Age of diagnosis of MDD was available for 1,316 cases and MDD duration derived as described above.

ELSA: ELSA is comprised of 12,003 individuals at wave 1 that were collected from the English population. Participants were eligible if they were aged 50 or over. Genotype data was available for 7,412 unrelated individuals which was reduced to 7,230 after QC. 757 MDD cases (13.1%) and 5,009 controls had available genotype data in wave 1 of the data. Wave 1 was selected for analysis as this had the largest sample size of MDD cases (males=2,662, females=3,196). Episode data and age of

onset of MDD was not available in ELSA therefore we could not infer recurrent MDD status or MDD duration.

LD score regression

Only SNPs that overlapped with HapMap Project Phrase 3 SNPs were included for calculation of SNP heritability and genetic correlations and the following thresholds were applied; INFO > 0.9, MAF > 1%, missingness of 0 and strand-ambiguous SNPs were excluded. For further details on this method please see original paper (B Bulik-Sullivan et al., 2015b; BK Bulik-Sullivan et al., 2015a). The intercept in this analysis was not constrained and multiple comparison testing was completed utilising false discovery rate (FDR).

Polygenic Profiling

To generate PRS in each cohort, SNPs were excluded on the following thresholds; MAF < 1%, Hardy Weinberg equilibrium $p>1x10^{-6}$, missingness per individual <1%, missingness per SNP <1%. Principle components were generated utilising the genome-wide complex trait analysis (GCTA) tool (Yang, Lee, Goddard, & Visscher, 2011) to control for population stratification and strand-ambiguous SNPs were removed. Clump based LD pruning was performed (r^2 0.25, 300kb window). For further details on this method please see original paper (International Schizophrenia Consortium et al., 2009).

Taylor Series Transformation

The Taylor series transformation (Cortes et al., 2013) is a method of converting a linear beta to an odds ratio (OR) on the liability scale. It uses the formula $OR = \frac{P+beta/(1-P-beta)}{P/(1-P)}$, where P represents the prevalence of the trait (in this case MDD) in the general population and beta is the beta coefficient from the linear association model.

Mendelian Randomisation (MR)

Genetic variants

Instrumental variables (IVs) were constructed of SNPs that reached genome-wide significant in the ENIGMA GWAS. As hippocampal volume was the only brain structure to demonstrate nominally significant genetic correlation, MR analysis was only completed on this subcortical structure. Two SNPs reached genome-wide significance in the original GWAS; rs61921502 and rs7294919. These SNPs were not available in the MDD PGC GWAS summary statistics and therefore the SNPs in highest LD available in both datasets (ENIGMA and PGC) were selected. rs17765551 was in LD with rs61921502 (R^2=0.51) and rs77956314 was in LD with rs7294919 (R^2=0.86).

ENIGMA SNPs (rs61921502 and rs7294919) were present in both GS:SFHS and UK Biobank however not in ELSA. This dataset was therefore excluded. The genetic variants were extracted using PLINK (Purcell et al., 2007) and coded as 0,1 or 2. These variants were then carried forward into an association analysis.

Association in GS:SFHS and UK Biobank

Following the same model used to measure the association between PRS and MDD, variant – exposure association was measured using mixed linear models in AS-Reml-R in GS:SFHS and logistic regression in UK Biobank. Fixed-effect meta-analysis between the 2 datasets for each SNP association was conducted using the 'meta' package in R.

Mendelian Randomisation (MR) analysis

We conducted MR analysis using the method outline by Bowden et al., (2015) which utilises 2 techniques; the Inverse Variance Weighted (IVW) method and MR-Egger regression (Bowden, Davey Smith, & Burgess, 2015). MR makes three assumptions of instrumental variables (IVs); (1) that the IVs are associated with the exposure, (2) that the IVs are not associated with any confounders and (3) that the association between outcome and exposure is conditional on the IVs (e.g. the IVs do not have a direct effect on the outcome). Any deviation from these assumptions would mean the IVs are invalid. Whilst the IVW method considers that the assumptions are true, MR-Egger regression relaxes these assumptions to account for directional pleiotropy. It uses a weaker assumption known as the Instrument Strength Independent of Direct

effect (InSIDE) which is the condition that the correlation between IVs – exposure and direct effect of IVs – outcome is zero. If the intercept differs from zero, then the model contains invalid IVs and the IVW estimate will be biased.

References

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Supplemental Table S1. Power analyses.

a)

	GCTA-GREML Power Calculator	
	Simulation rG= 0.5	Measured rG
Accumbens	0.81	0.060
Amygdala	-	-
Caudate	1.00	0.13
Hippocampus	0.96	0.93
ICV	0.98	0.20
Pallidum	0.98	0.051
Putamen	1.00	0.22
Thalamus	0.94	0.097

b i)

	PRS in association of own structure		MDD PRS Meta-Analysis	
	PRS thresholds	Power	Covariance	Power
Accumbens	0.01	0.0560	5%	0.0503
	0.05	0.0659		
	0.1	0.0730		
	0.5	0.0921		
	1	0.0946		
Amygdala	0.01	0.0537	50%	0.248
	0.05	0.0602	25%	0.0980
	0.1	0.0649	10%	0.0575
	0.5	0.0779		
	1	0.0797		
Caudate	0.01	0.159	8%	0.113
	0.05	0.262		
	0.1	0.318		
	0.5	0.432		
	1	0.444		
Hippocampus	0.01	0.0696	46%	0.371
	0.05	0.0970		
	0.1	0.115		
	0.5	0.161		
	1	0.167		
ICV	0.01	0.0827	12%	0.163
	0.05	0.124		
	0.1	0.151		
	0.5	0.215		
	1	0.223		
Pallidum	0.01	0.0857	1%	0.0504
	0.05	0.130		

	0.1	0.159		
	0.5	0.226		
	1	0.234		
Putamen	0.01	0.227	10%	0.157
	0.05	0.365		
	0.1	0.434		
	0.5	0.558		
	1	0.569		
Thalamus	0.01	0.0654	8%	0.0662
	0.05	0.0879		
	0.1	0.103		
	0.5	0.142		
	1	0.147		

ii)

	Meta-analysis	
	Covariance	Power
Recurrent	50%	0.264
	25%	0.102
	10%	0.0581
Episodes	50%	0.532
	25%	0.175
	10%	0.0693
Duration	50%	0.0957
	25%	0.0612
	10%	0.0518
Age of Onset	50%	0.0957
	25%	0.0612
	10%	0.0518

GCTA-GREML power calculator was used to calculate (a) power for genetic correlation at the measured correlation and at a simulated rg=0.5. AVENGEME was used to predict power of PRS in (b i) the meta-analysis between subcortical structures and MDD and in prediction of own structure in UK Biobank (b ii) the meta-analysis between depression traits and hippocampal volume. Covariance was measured, were possible, utilising LD score regression between ENIGMA subcortical structures and PGC MDD. If covariance could not be calculated, covariances of 50%, 25% and 10% have been reported. SNP heritability values were calculated from LD score regression were possible, otherwise values from published sources were used. Abbreviations: GCTA, genome-wide complex trait analysis; GREML, genomic-relatedness-matrix restricted maximum-likelihood; rG, genetic correlation.

Supplemental Table S2. Linear regression analysis of polygenic score for regional brain volume and their respective volume in UK Biobank.

		Respective total brain region volume	
	PRS Threshold	***P* value**	**Stats**
Nucleus accumbens	*0.01*	0.0695	β= 0.0516 R^2= 0.00260
	0.05	0.239	β= 0.0333 R^2= 0.00110
	0.1	0.412	β= 0.0237 R^2= 0.000531
	0.5	0.334	β= 0.0274 R^2= 0.000735
	1	0.343	β= 0.0270 R^2= 0.000708
Amygdala	*0.01*	0.902	β= -0.00378 R^2= $1.36x10^{-5}$
	0.05	0.992	β= -0.000303 R^2= $8.5x10^{-8}$
	0.1	0.703	β= -0.0121 R^2= 0.000131
	0.5	0.948	β= 0.00212 R^2= $3.79x10^{-6}$
	1	0.828	β= 0.00707 R^2= $4.24x10^{-5}$
Caudate nucleus	*0.01*	**0.00336**	**β= 0.0795** **R^2= 0.00586**
	0.05	**0.000185**	**β= 0.107** **R^2= 0.00950**
	0.1	**0.000151**	**β= 0.113** **R^2= 0.00975**
	0.5	**0.000108**	**β= 0.117** **R^2= 0.0102**
	1	**0.000184**	**β= 0.115** **R^2= 0.00950**
Hippocampus	*0.01*	**0.00417**	**β= 0.0806** **R^2= 0.00605**
	0.05	0.161	β= 0.0420 R^2= 0.00145
	0.1	0.0704	β= 0.0536 R^2= 0.00242
	0.5	0.236	β= 0.0341 R^2= 0.00104
	1	0.157	β= 0.0407 R^2= 0.00148
ICV	*0.01*	**0.0418**	**β= 0.0509** **R^2= 0.00267**
	0.05	**0.0134**	**β= 0.0649** **R^2= 0.00392**

	0.1	**0.00204**	**β= 0.0822** **R^2= 0.00609**
	0.5	**0.000351**	**β= 0.0954** **R^2= 0.00817**
	1	**0.000388**	**β= 0.0947** **R^2= 0.00805**
Pallidum	*0.01*	0.902	β= -0.00353 R^2= $1.14x10^{-5}$
	0.05	0.965	β= -0.00131 R^2= $1.44x10^{-6}$
	0.1	0.617	β= 0.0150 R^2= 0.000186
	0.5	0.131	β= 0.0474 R^2= 0.00170
	1	0.163	β= 0.0437 R^2= 0.00145
Putamen	*0.01*	**0.00163**	**β= 0.0758** **R^2= 0.00492**
	0.05	**$1.56x10^{-5}$**	**β= 0.118** **R^2= 0.00921**
	0.1	**0.000118**	**β= 0.108** **R^2= 0.00733**
	0.5	**0.00451**	**β= 0.0878** **R^2= 0.00400**
	1	**0.00498**	**β= 0.0873** **R^2= 0.00391**
Thalamus	*0.01*	**0.00602**	**β= 0.0519** **R^2= 0.00286**
	0.05	**0.00124**	**β= 0.0630** **R^2= 0.00395**
	0.1	**0.00464**	**β= 0.0556** **R^2= 0.00304**
	0.5	**0.00513**	**β= 0.0549** **R^2= 0.00300**
	1	**0.00430**	**β= 0.0561** **R^2= 0.00309**

Five P value thresholds were used; 0.01, 0.05, 0.1, 0.5 and 1. Significant values (P<0.05) are shown in bold.

Supplemental Table S3. Unadjusted and FDR corrected *P* values for LD score regression genetic correlation with MDD.

	Nucleus accumbens	**Amygdala**	**Caudate nucleus**	**Hippocampus**	**ICV**	**Pallidum**	**Putamen**	**Thalamus**
Unadjusted P value	0.828	NA	0.562	**0.0213**	0.460	0.961	0.404	0.648
FDR corrected P value	0.961	NA	0.907	0.149	0.907	0.961	0.907	0.907

The genetic correlation between hippocampus volume and MDD does not withstand FDR correction for multiple testing. Significant values ($P<0.05$) are shown in bold. Abbreviations: FDR, false discovery rate.

Supplemental Table S4. Mixed model analysis of Subcortical volumetric and ICV PRS with MDD in all 3 cohorts.

PRS	Study	P value	beta	s.e.
Nucleus accumbens	**GS**	0.485	-0.0181	0.0218
	UKB	0.284	-0.0151	0.0141
	ELSA	0.659	0.0175	0.0395
Amygdala	**GS**	0.327	-0.0285	0.0217
	UKB	0.962	0.000694	0.0146
	ELSA	0.208	-0.0500	0.0398
Caudate nucleus	**GS**	0.246	0.0197	0.0224
	UKB	0.396	-0.0135	0.0159
	ELSA	0.114	-0.0630	0.0398
Hippocampus	**GS**	0.782	-0.00299	0.0211
	UKB	0.601	-0.00732	0.0140
	ELSA	0.571	-0.0222	0.0392
ICV	**GS**	0.395	0.0179	0.0225
	UKB	0.280	-0.0160	0.0148
	ELSA	0.0995	0.0652	0.0396
Pallidum	**GS**	0.752	-0.00808	0.0221
	UKB	0.250	0.0178	0.0155
	ELSA	0.658	0.0176	0.0398
Putamen	**GS**	0.303	-0.0246	0.0218
	UKB	0.215	0.0231	0.0186
	ELSA	0.695	-0.0161	0.0410
Thalamus	**GS**	0.451	-0.0192	0.0219
	UKB	0.836	0.00293	0.0142
	ELSA	0.222	-0.0482	0.0395

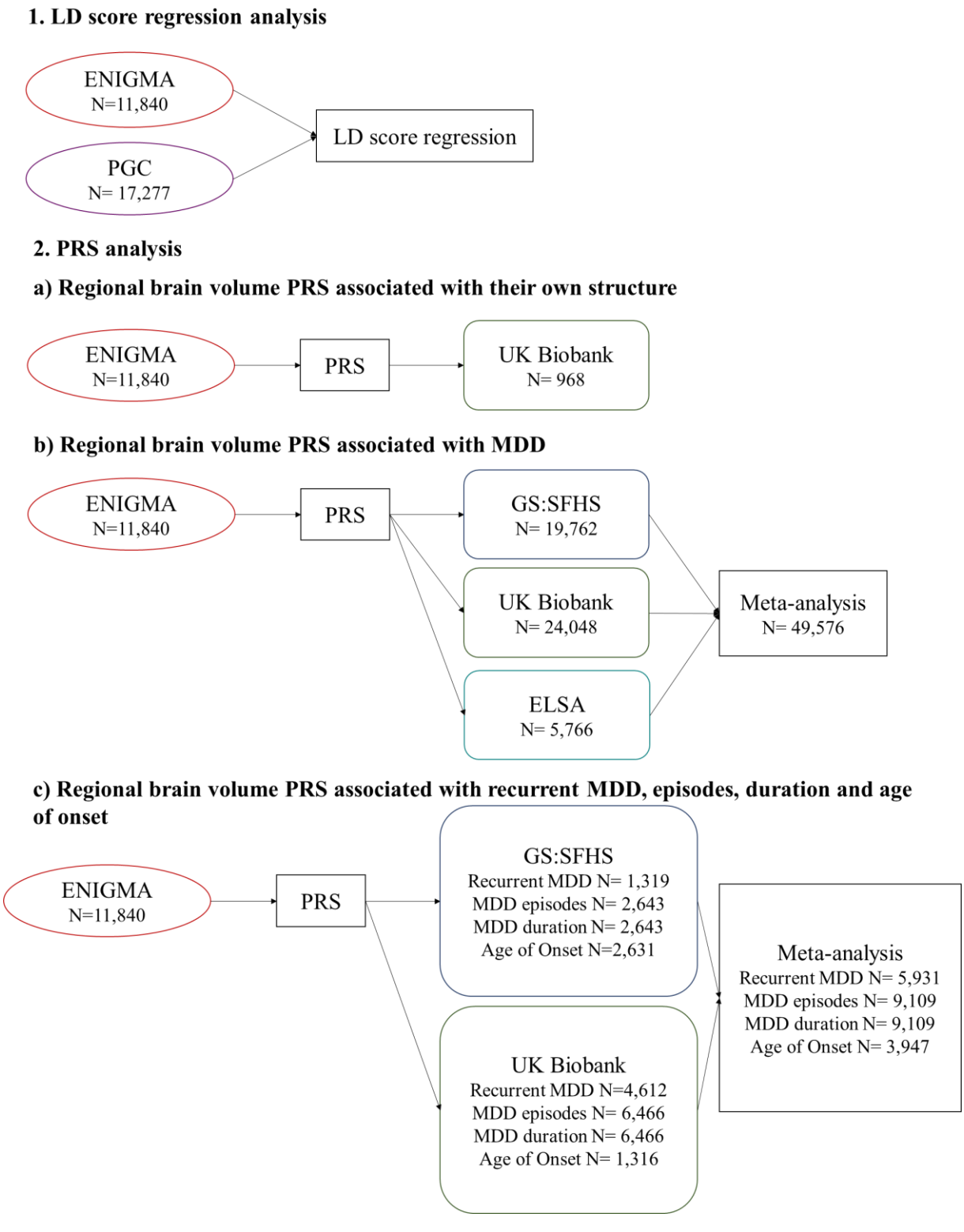
Best *P* value threshold for PRS were carried forward in this analysis; Nucleus accumbens=0.01, Amygdala= 0.1, Caudate nucleus=0.5, Hippocampus=0.01, ICV=0.5, Pallidum=0.5, Putamen=0.1, Thalamus=0.05. No PRS demonstrated a significant association with MDD in any cohort. Abbreviations: PRS, polygenic risk scores; ICV, intracranial volume; GS, Generation Scotland; UKB, UK Biobank; ELSA, English Longetudinal Study of Ageing.

Supplemental Table S5. MR analysis using IVW method.

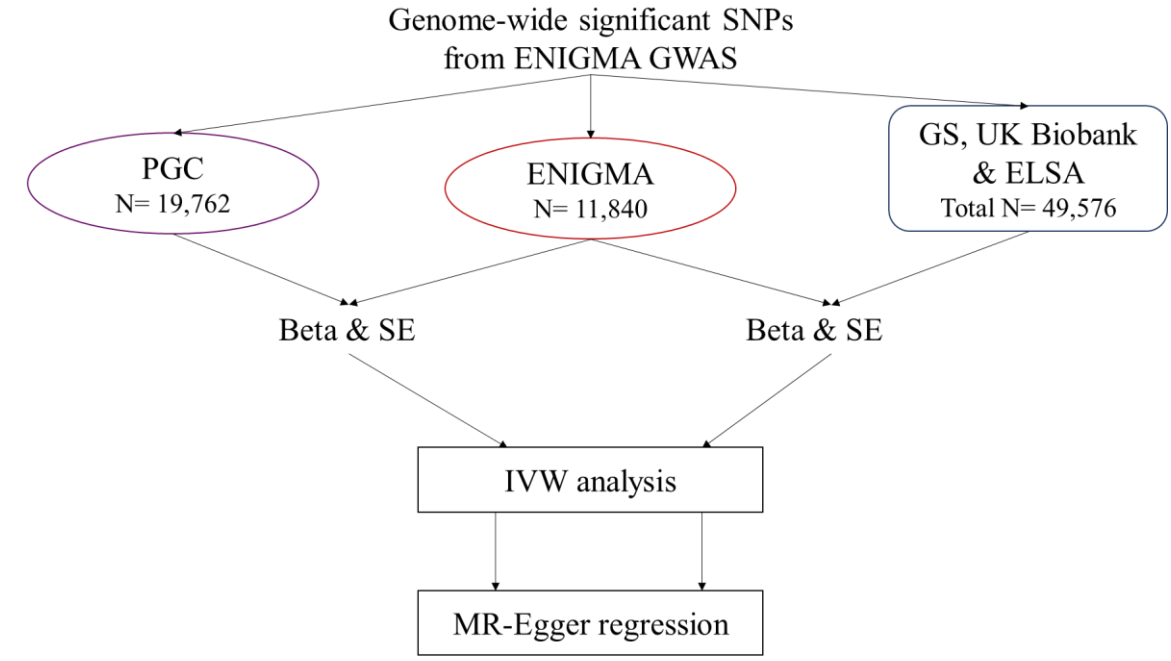
	Inverse-Variance Weighted method		
	***P* value**	**Beta**	**SE**
Hippocampus-GS&UKB MDD	0.361	0.0100	0.005
Hippocampus-PGC MDD	0.077	0.004	0.007

Abbreviations: GS, Generation Scotland; UKB, UK Bioabank; PGC, Psychiatric Genomics Consortium; Standard Error.

Figure S1. Flow chart of methodology.



3. Mendelian randomisation analysis



4. BUHMBOX

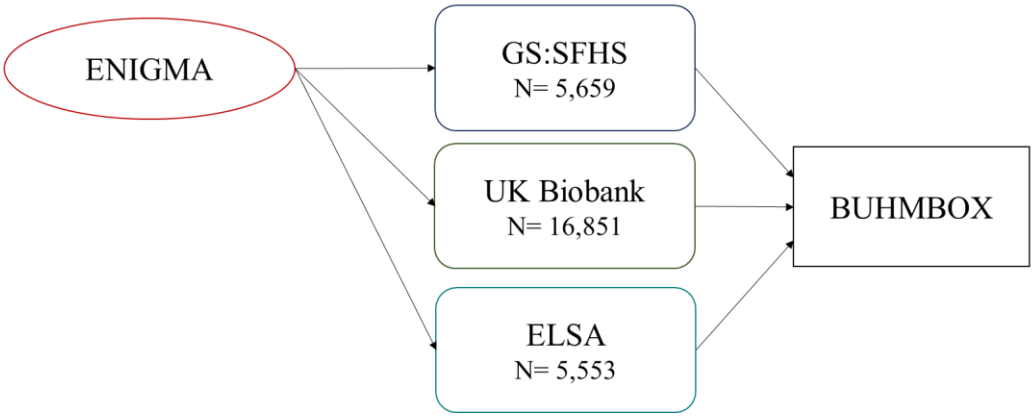
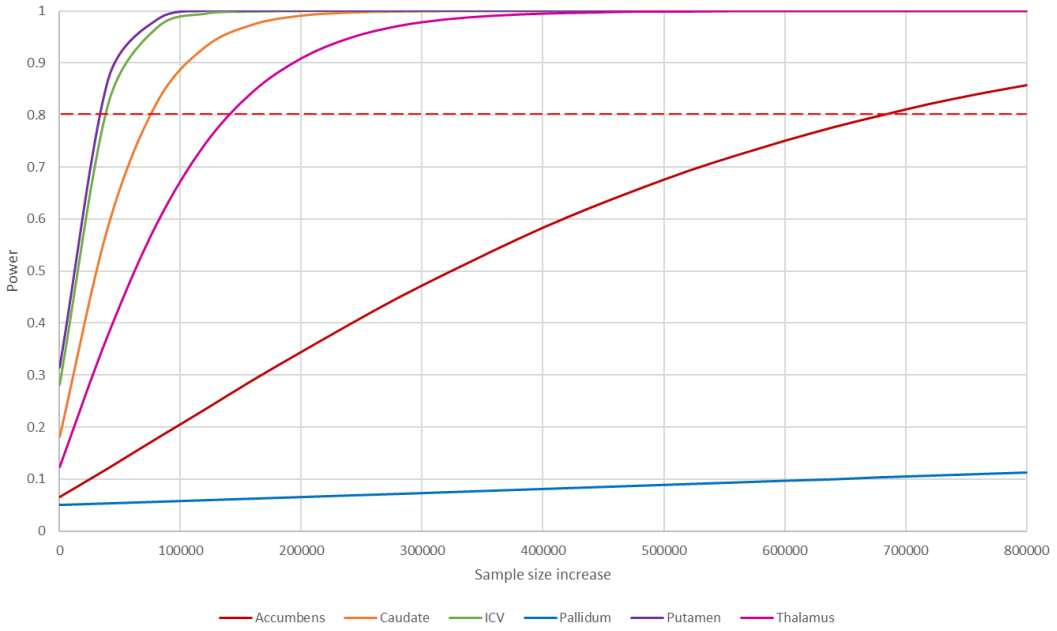
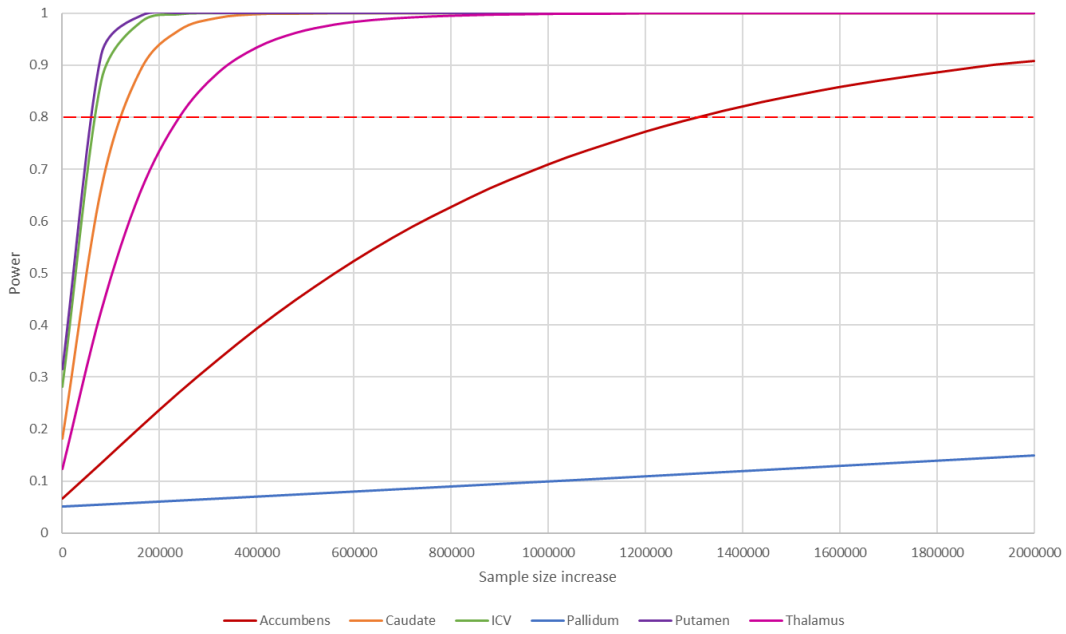


Figure S2. Power curves for genetic correlation analysis a) increasing sample size for subcortical structures only and b) increasing sample size for MDD only

a)



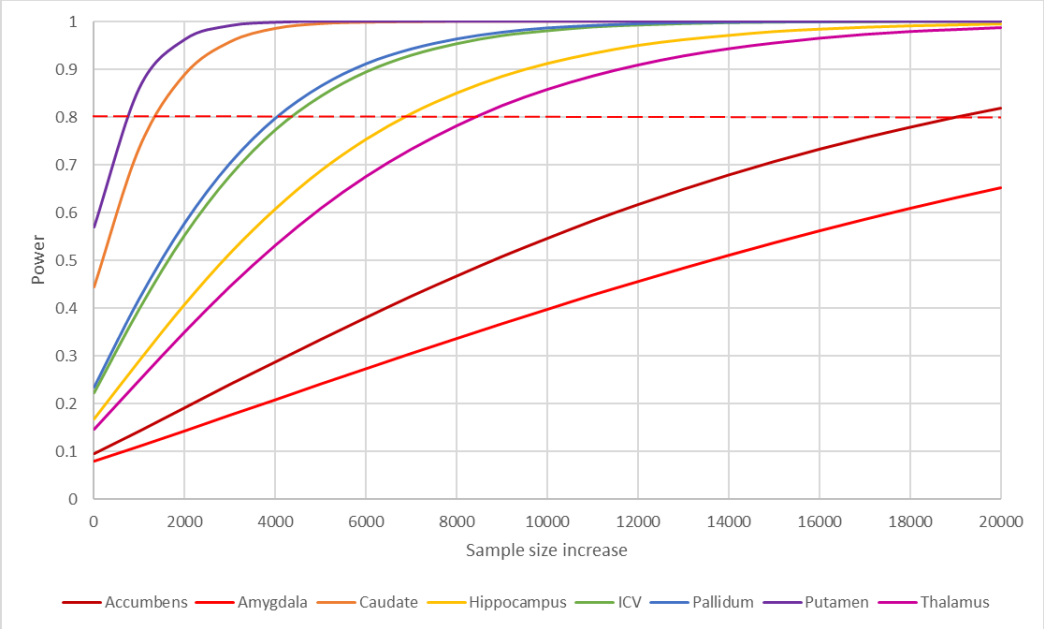
b)



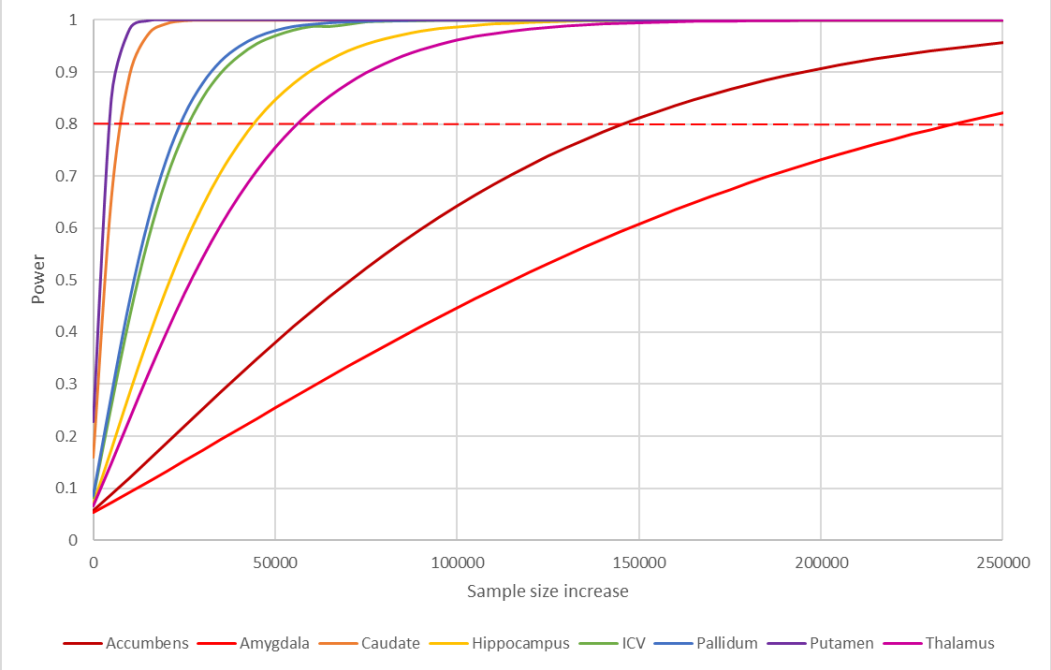
Power curves were calculated with starting point 0 as the sample size in our analysis. For increasing the sample for MDD it was assumed the ratio of cases and controls remained the same therefore a sample increase of 1000 would include 500 cases and 500 controls. Hippocampus was not included as this had adequate power.

Figure S3. Power curves for PRS analyses a) subcortical PRS associated with their respective structures at a P value threshold of (i) 1 and (ii) 0.01 and b) subcortical PRS associated with recurrent MDD, age of onset, episodes and duration.

a) i)



ii)



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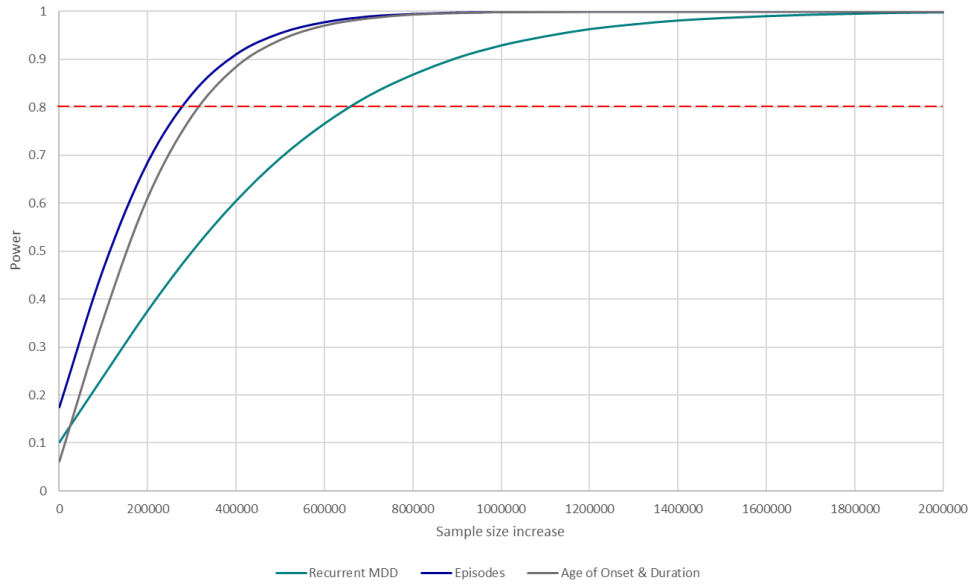
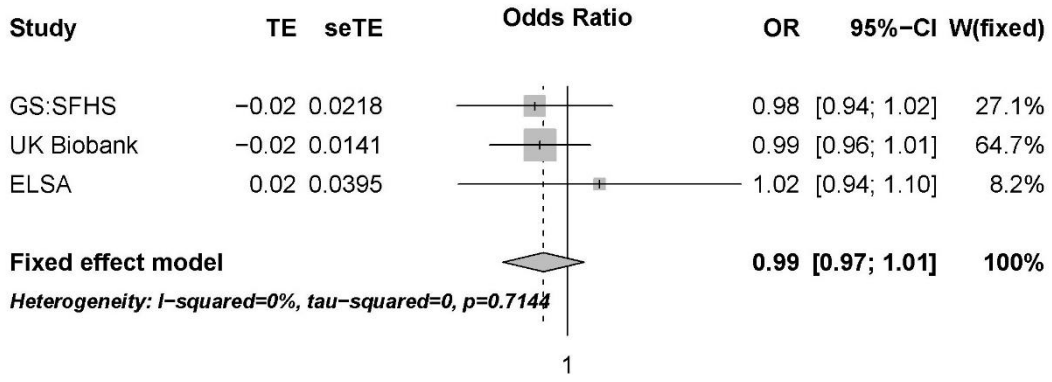


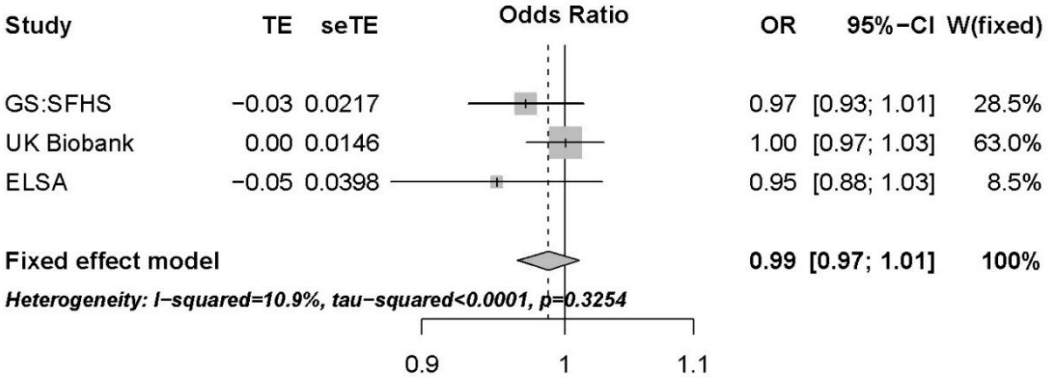
Power curves assumed training sample size (ENIGMA subcortical volumes) remained constant and sample size for the target data set was increased from point 0 (the sample at which this analysis was conducted). Amygdala, recurrent MDD, episodes, age of onset and duration were all assumed to have a genetic correlation of 25%.

Figure S4. Meta-analysis forest plots of mixed model analysis of subcortical volume PRS and MDD in Generation Scotland: Scottish Family Health Study (GS:SFHS), UK Biobank and English Longitudinal Study of Ageing (ELSA).

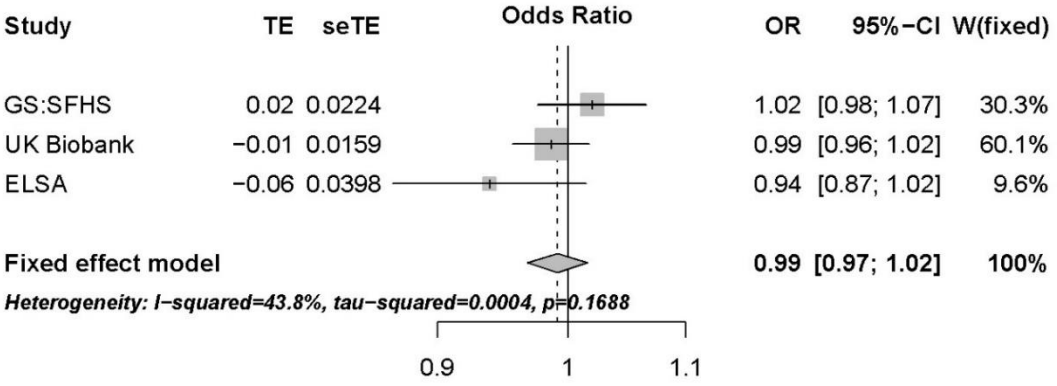
S3. a) Nucleus Accumbens PRS



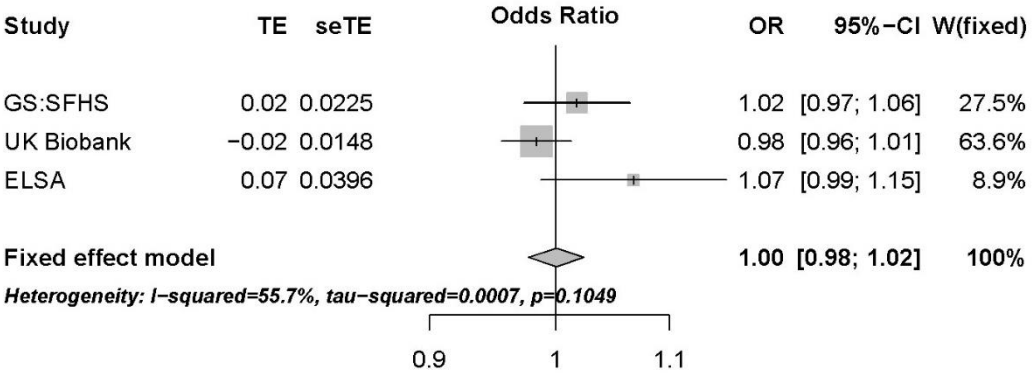
b) Amygdala PRS



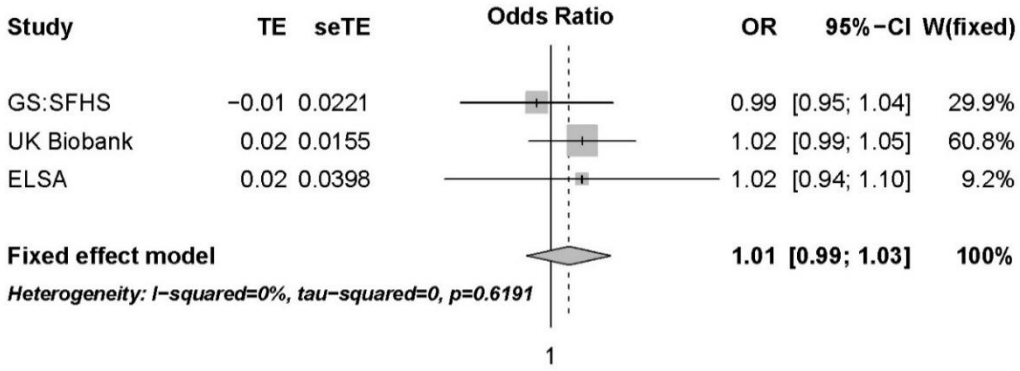
c) Nucleus caudate PRS



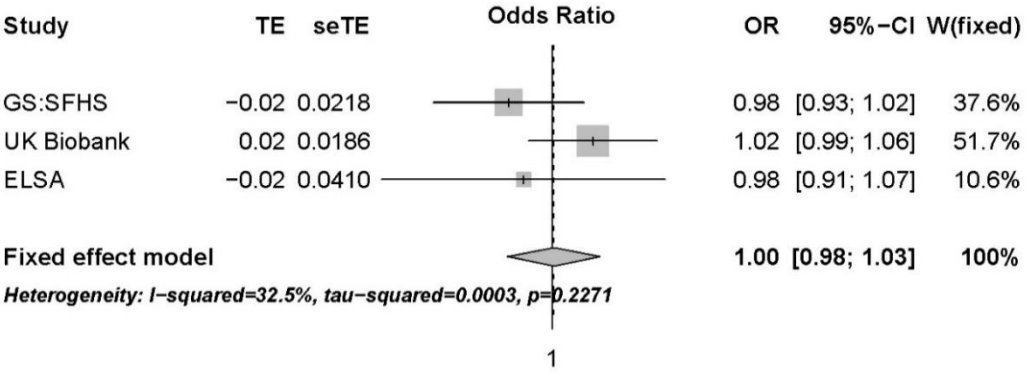
d) ICV PRS



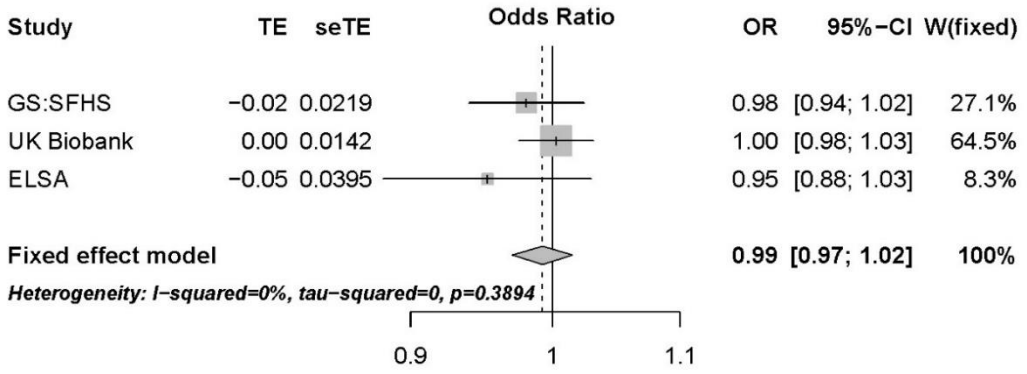
e) Pallidum PRS



f) Putamen PRS



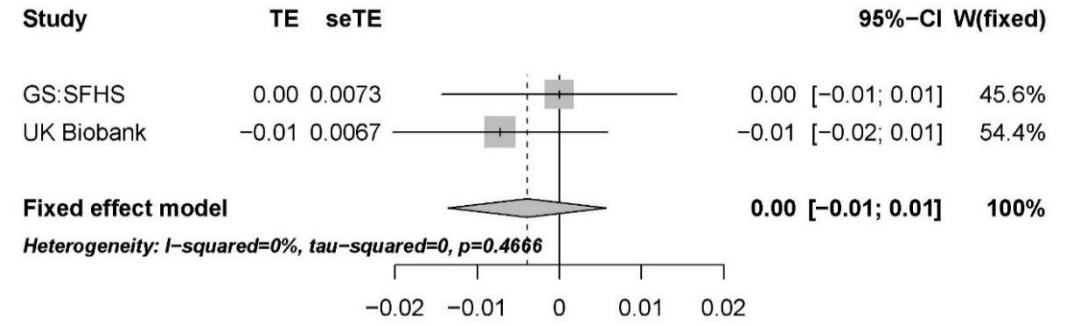
g) Thalamus PRS



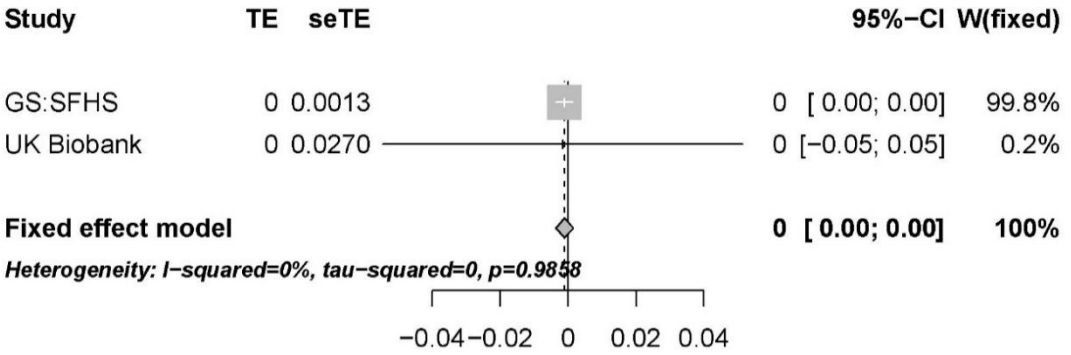
No heterogeneity was reported between cohorts but no regional brain PRS is significantly associated with MDD. Abbreviations: TE; treatment effect (regression beta's); seTE, standard errors; OR, odds ratio; CI, confidence intervals; W(fixed), weight of individual studies in fixed effect meta-analysis.

Figure S5. Meta-analysis forest plots of mixed model analysis of hippocampal volume PRS and MDD episodes, MDD duration and age of onset in GS:SFHS and UK Biobank.

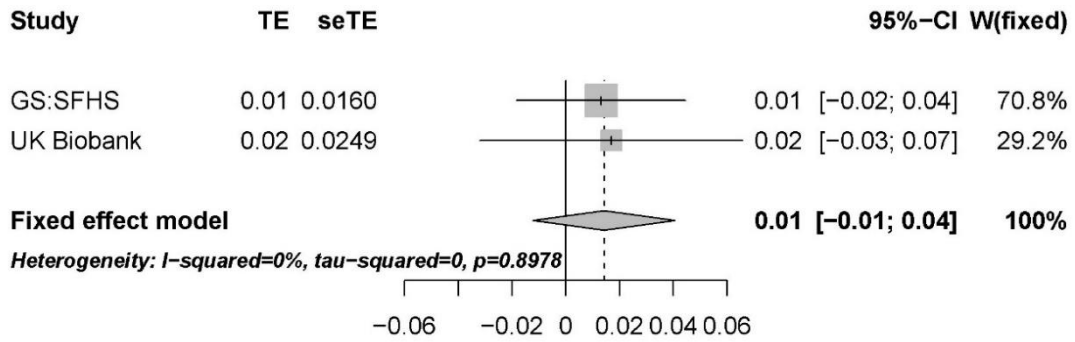
S4. a) MDD episodes



b) MDD duration



c) Age of Onset



No heterogeneity was reported between cohorts but hippocampal volume PRS was not significantly associated with number of MDD episodes (a), MDD duration (b) or age of onset (c). Abbreviations: TE; treatment effect (regression beta's); seTE, standard errors; CI, confidence intervals; W(fixed),weight of individual studies in fixed effect meta-analysis.

Appendix 2. Supplementary Material to Section 4.2.

Supplementary material for the manuscript by Wigmore et al "Genome-wide association study of antidepressant treatment resistance in a population-based cohort using health service prescription data with meta-analysis with GENDEP."

Contents:

Supplemental Methods

Cohort description

Generation Scotland: Scottish Family Health Study (GS:SFHS): GS:SFHS is a cohort of 24,080 participants, aged between 18 and 98 (mean age=47.6, s.d.=15.4), recruited from Scottish medical practices between 2006 and 2011. Participants were recruited at random after individuals were identified via their Community Health Number and it was a requirement for participants to also have a first-degree relative over the age of 18 willing to participate. Data on mood, cognition and personality were obtained through questionnaires and genotype data was obtained for 20,032 individuals (after QC n=19,994). Schizophrenia and bipolar individuals were identified through record linkage to the Scottish Morbidity Record, general hospital inpatient, general hospital outpatient and psychiatric inpatient/outpatient data (SMR00, SMR01, SMR04). Prescription data was available through the Prescribing Information System (PIS) which records all Scottish NHS prescriptions for payments for medications prescribed by general practitioners (GPs), nurses, pharmacists and hospitals where the medication was dispensed in the community (hospital dispensed prescriptions are not included). Records were available for the period between April 2009 and February 2015.

Prescription records in GS:SFHS

Information available from prescription records included the dose of the prescription given, amount of drug dispensed and prescription instructions. From this, a daily dose was calculated based on the dose of the prescription and the instructions for daily intake. Prescription instructions were sometimes given as a range, where this was the case, e.g. “1-2 tablets per day”, the lower range was selected to calculate daily dose to ensure an individual was taking at least an adequate dose. For calculations regarding duration of prescription, the upper range was selected to ensure an adequate duration. Duration on the antidepressant was calculated based on the dispensed amount and daily dose. Entries were then distinguished into prescription episodes. i.e. periods where the same antidepressant had been prescribed continuously for at least 6 weeks. All

prescription episodes that fell below this were excluded. Duration of a drug was calculated additively based on a continuous prescription. Date of drug dispense was available at month and year only therefore when determining a continuous prescription time point a 31 day margin was given. British National Formulary (BNF) guidelines differ for elderly patients (age>64 years) therefore age at prescription was considered when excluding those below the minimum dose (a total of 29,942 entries excluded). BNF guidelines recommend that at least 4 weeks of treatment should be completed before considering switching the antidepressant due to lack of efficacy however we applied a stricter threshold of at least 6 weeks.

Defining stages of resistance (SR)

Our definition is loosely based on the Massachusetts General Hospital's staging (MGH-S) method(Fava, 2003) which has been previously recommended as a useful research tool(Hazari, Christmas, & Matthews, 2013). However, this method has met criticism for arbitrarily weighting stages on dose optimisation/drug augmentation and electroconvulsive therapy (ECT)(Berlim & Turecki, 2007). Therefore, our staging method was purely based on antidepressant switching given an adequate duration and dose.

Cognitive and personality traits in GS:SFHS

To obtain 'g' (general intelligence) a principle component analysis (PCA) was conducted in cognitive tests. 4 Cognitive tests were assessed; digit symbol coding, vocabulary, verbal fluency and logical memory. Digit symbol coding was measured using the Wechsler digit symbol substitution task(Wechsler, 1998) to measure participants processing speed. For vocabulary, the Mill Hill vocabulary scale(Raven, Raven, & Court, 1998) (combined junior and senior synonyms) was used and, to test executive functioning, a 1 minute letter-based verbal fluency test was used with letters C, F and L(Lezak, 1995). Logical memory was assessed by summation of the immediate and delayed score for recall of a paragraph in the Wechsler logical memory test(Wechsler, 1998). The first unrotated principle component was utilised as 'g' and explained 41% of the total variance between the 4 tests.

Neuroticism and extraversion were assessed using the Eysenck Personality Questionnaire Short Form-Revised(Eysenck, 1991) with a score ranging 0-12. Schizotypal personality and mood disorders were measured by the schizotypal personality questionnaire (SPQ) version B(Raine & Benishay, 1995) and mood disorder questionnaire (MDQ)(Hirschfeld, 2002), respectively. Higher scores indicated higher expression of the personality trait. The general health questionnaire 28 (GHQ)(Goldberg & Hillier, 1979) was used as a measure of psychological distress with 28 questions scored from 0 ("not at all") to 3 ("much more than usual").

To measure social deprivation, the Scottish Index of Multiple Deprivation (SIMD)(Payne & G, 2012) was used. In brief, this ranks areas according to crime, housing, education, income, health and geographical access. Each area then receives a rank from 1 to 6,505 which is then converted to a quintile, 1 being most deprived and 5 being least deprived. Education was scored as years in full-time education and ranged from 0-10.

Construction of Genetic Relationship Matrices (GRMs)

Two GRMs were fitted utilising the method created by Zaitlen *et al.* (2013) The first GRM included pairwise relationship coefficients for all individuals and the second included off-diagonal elements of pairs of individuals who had a relationship coefficient < 0.05 set to 0. This therefore excluded pairs of individuals that have a most recent common ancestor of approximately four generations distant, assuming no inbreeding. This method has been demonstrated to account for potential upward biases due to excessive relationships, thus allowing the inclusion of closely and distantly related individuals in genetic analyses(Zaitlen et al., 2013).

Taylor series transformation

The Taylor series transformation(Cortes et al., 2013) is used to convert a linear beta to an odds ratio (OR) using the formula $OR = \frac{P+beta/(1-P-beta)}{P/(1-P)}$, where P indicates the trait prevalence in the general population and beta is the beta coefficient from the linear association model.

Gene and gene-set enrichment using MAGMA

MAGMA(de Leeuw, Mooij, Heskes, & Posthuma, 2015) uses multiple regression to identify gene-sets that are associated with the phenotype while accounting for linkage disequilibrium (LD) between markers by combing SNP *P* values. The LD reference panel used was the European-ancestry subjects from 1000 Genomes Project(Abecasis et al., 2012). Both individual level genotype data and summary statistics can be used as input, however individual level genotype data are considered the preferred choice.

Polygenic risk scores (PRS)

PRS is an additive SNP score constructed per individual weighted by the effect size from a training set (GWAS summary statistics). Due to the application of linkage disequilibrium (LD) pruning, PRS were constructed utilising genotyped data rather than imputed. Data was quality controlled (QC) using the following thresholds; minor allele frequency (MAF) > 1%, Hardy Weinberg equilibrium (HWE) $p>1x10^{-6}$, missingness per individual <1%, missingness per SNP <1%. Clump based pruning was used (r^2=0.25, 300kb window) and strand-ambiguous SNPs were removed. Multi-dimensional scaling (MDS) components were created in PLINK(Purcell et al., 2007) to control for population stratification. Further details on this method can be found in the original paper(International Schizophrenia Consortium et al., 2009).

Trait variance explained by the PRS was calculated using (var($x \times \beta$))/var(y), where x was the standardized PGS, β was the corresponding regression coefficient and y was the phenotype(Nakagawa & Schielzeth, 2013)

Psychiatric Genomics Consortium (PGC) MDD GWAS summary statistics

Unpublished data was used to construct PRS in GS:SFHS using the latest PGC release for MDD. The sample consisted of 51,865 MDD cases and 112,200 controls.

Genetic Correlation

Genetic correlations (r_g) is defined as $r_g = \frac{cov_G}{\sqrt{V_{Gi} * V_{Gj}}}$ where cov_G is the additive covariance, V_{Gi} is the additive variance of the personality or cognitive variable and V_{Gj} is the additive variance of TR or SR. Significance was calculated using the likelihood

ratio test to compare the model against a null model (model assuming no covariance between the traits).

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Supplemental Table S1. Full list of antidepressants being prescribed in GS:SFHS.

Antidepressant Class	Prescription name	Frequency
Selective Serotonin Reuptake Inhibitors (SSRI)	CITALOPRAM	1555
	ESCITALOPRAM	117
	FLUOXETINE	1130
	FLUVOXAMINE MALEATE	2
	PAROXETINE	105
	SERTRALINE	584
	Total	**3493**
Serotonin Noradrenaline Reuptake Inhibitors (SNRI)	DULOXETINE	114
	VENLAFAXINE	224
	Total	**338**
Serotonin Antagonist and Reuptake Inhibitors (SARI)	TRAZODONE HYDROCHLORIDE	66
	Total	**66**
Noradrenaline Reuptake Inhibitors (NRI)	REBOXETINE	4
	Total	**4**
Tricyclic Antidepressants (TCA)	AMITRIPTYLINE	178
	CLOMIPRAMINE HYDROCHLORIDE	25
	DOSULEPIN HYDROCHLORIDE	48
	DOXEPIN	5
	IMIPRAMINE HYDROCHLORIDE	14
	LOFEPRAMINE	39
	NORTRIPTYLINE	7
	TRIMIPRAMINE	3
	Total	**319**
Tetracyclic Antidepressants (TeCA)	MIRTAZAPINE	453
	Total	**453**
Monoamine Oxidase Inhibitors (MAOI)	MOCLOBEMIDE	6
	PHENELZINE	6
	TRANYLCYPROMINE	3
	Total	**15**
Melatonergic Antidepressants	AGOMELATINE	2
	Total	**2**

Supplemental Table S2. Description of GS:SFHS subjects included in TR study.

Variable	TR (n=250)	Non-TR (n=3,202)
Age	45.04±13.96	47.32±13.92
Gender (F/M)	195/55	2335/867
Total antidepressants taken	3 for 186 individuals (67.2%) 4 for 46 individuals (18.4%) 5 for 13 individuals (5.2%) 6 for 1 individuals (0.4%) 7 for 2 individuals (0.8%) 8 for 2 individuals (0.8%)	1 for 2557 individuals (79.9%) 2 for 645 individuals (20.1%)
N of previous SSRI treatments	0 for 8 individuals (3.2%) 1 for 53 individuals (21.2%) 2 for 118 individuals (47.2%) 3 for 69 individuals (27.6%) 4 for 2 individuals (0.8%)	0 for 539 individuals (16.8%) 1 for 2333 individuals (72.9%) 2 for 330 individuals (10.3%)
N of previous TCA treatments	0 for 200 individuals (80%) 1 for 41 individuals (16.4%) 2 for 9 individuals (3.6%)	0 for 2993 individuals (93.5%) 1 for 206 individuals (6.4%) 2 for 3 individuals (0.1%)
N of previous MAOI antidepressants	0 for 244 individuals (97.6%) 1 for 6 individuals (2.4%)	0 for 3193 individuals (99.7%) 1 for 9 individuals (2.8%)
N of previous treatments with other antidepressants	0 for 202 individuals (80.8%) 1 for 139 individuals (55.6%) 2 for 51 individuals (20.4%) 3 for 12 individuals (4.8%)	0 for 2645 individuals (82.6%) 1 for 524 individuals (16.4%) 2 for 33 individuals (1.0%)

Supplemental Table S3. Description of GENDEP subjects included in TR study.

Variable	TR (n=109)	Non-TR (n=668)
Age	43.54±11.15	41.90±11.62
Gender (F/M)	73/36	411/257
Baseline MADRS	30.38±6.62	28.58±6.74
Baseline HAMD	23.03±5.01	21.66±5.26
Age at onset	32.10±11.88	31.86±10.38
N of previous episodes	1.95±0.74	1.69±0.66
Antidepressant during longitudinal trial	Escitalopram: 50 Nortriptyline: 59	Escitalopram: 391 Nortriptyline: 277
Antidepressant switch during longitudinal trial (yes/no)	71/38	35/633
N of previous SSRI treatments	0 for 50 individuals (45.9%) 1 for 39 individuals (35.8%) 2 for 17 individuals (15.6%) 3 for 3 individuals (2.8%)	0 for 498 individuals (74.6%) 1 for 145 individuals (21.7%) 2 for 19 individuals (2.8%) 3 for 6 individuals (0.9%)
N of previous TCA treatments	0 for 68 individuals (62.4%) 1 for 28 individuals (25.7%) 2 for 10 individuals (9.2%) 3 for 3 individuals (2.8%)	0 for 600 individuals (89.8%) 1 for 58 individuals (8.9%) 2 for 6 individuals (0.9%) 3 for 3 individuals (0.4%) 5 for 1 individuals (0.1%)
N of previous treatments with dual antidepressants	0 for 89 individuals (81.7%) 1 for 19 individuals (17.4%) 2 for 1 individuals (0.9%)	0 for 637 individuals (95.4%) 1 for 31 individuals (4.6%)
N of previous MAOI treatments	0 for 104 individuals (95.4%) 1 for 5 individuals (4.6%)	0 for 656 individuals (98.2%) 1 for 12 individuals (1.8%)
N of previous treatments with other antidepressants	0 for 86 individuals (78.9%) 1 for 20 individuals (18.3%) 2 for 3 individuals (2.8%)	0 for 635 individuals (95.1%) 1 for 31 individuals (4.6%) 2 for 2 individuals (0.3%)
Previous antidepressant trial yes/no	77/32	281/387

Supplemental Table S4. Top MAGMA genes and gene-sets in treatment resistance (TR) and stages of resistance (SR) to antidepressants

GENE ENRICHMENT ANALYSIS

Treatment Resistance				**Stages of Resistance**			
Genes	***P* value**	**P_{FDR}**	**Effect Size**	**Genes**	***P* value**	**P_{FDR}**	**RSQ**
AASDHPPT	$5.81x10^{-5}$	0.465	8.0	ZFP28	$3.1x10^{-5}$	0.45	0.0082
PLOD2	$1.16x10^{-4}$	0.464	12.5	ZNF600	$1.7x10^{-4}$	0.54	0.0058
NEUROG3	$1.31x10^{-4}$	0.465	2.0	ZFR2	$4.5x10^{-4}$	0.51	0.0076

GENE-SET ENRICHMENT ANALYSIS

Treatment Resistance				**Stages of Resistance**			
Gene-sets	***P* value**	**Corrected *P***	**Beta**	**Gene-sets**	***P* value**	**Corrected *P***	**Beta**
IL-4 pathway	0.000485	0.347	0.803	Toll-like receptor signalling	0.00019	0.16	0.34
IL-3 pathway	0.000991	0.555	0.641	Fc epsilon RI-mediated signalling	0.00033	0.24	0.35
Pathogenic Escherichia coli infection	0.00153	0.701	0.348	FCER1 pathway	0.0066	0.41	0.46

Supplemental Table S6. Power analysis for GWAS of Treatment Resistance and Stages of Resistance for SNPs at a MAF of 1% and 5%

	Treatment Resistance		Stages of Resistance	
MAF	**OR**	**N cases**	**Beta**	**N**
0.01	1.1	225,675	0.1	87,102
	1.6	7,596	0.3	9,660
	2.1	2,656	0.5	3,465
0.05	1.1	47,244	0.1	18,138
	1.6	1,624	0.3	4,520
	2.1	579	0.5	706

Abbreviations: MAF, minor allele frequency; OR, odds ratio.

Appendix 3. Supplementary Material to Section 5.2.

Supplementary material for the manuscript by Wigmore et al "Antidepressant treatment resistance and the impact of neuroticism, psychological resilience and coping style."

Contents:

Supplemental Methods

Cohort description

Generation Scotland: Scottish Family Health Study (GS:SFHS): GS:SFHS is a family and population-based cohort that were recruited at random from medical practices. Individuals were recruited if they were aged 18 or over and had a first-degree relative willing to take part (age range=18-98, mean age=47.6, s.d.=15.4). At initial testing over 21,500 individuals were assessed for mood, cognition, personality and clinical measurements. DNA was also collected for 98% of the cohort (B. Smith et al., 2006; B. H. Smith et al., 2013). In 2014, a follow-up questionnaire was issued which included information on coping style and resilience (all of which were used in this study). Over 9,000 individuals returned these questionnaires (Navrady et al., 2017).

Record linkage in GS:SFHS

98% of the cohort consented to linkage to National Health Service (NHS) records which included the Prescribing Information System (PIS) and the Scottish Morbidity Record (SMR). Individuals with schizophrenia, schizoaffective and bipolar disorder were identified using the SMR, general hospital inpatient, general hospital outpatient and psychiatric inpatient/outpatient data (SMR00, SMR01, SMR04). Prescribing information was obtained through the PIS which is a record of all prescriptions issued through general practitioners (GPs), nurses, pharmacists and hospitals where the medication was dispensed in the community (hospital dispensed prescriptions are not included).

Defining antidepressant treatment resistance (TR)

TR was obtained by extracting all individuals in the cohort on antidepressants (**Table S1**). Prescribing information included the dose per prescription, the amount of drug prescribed and the instructions for intake. Instructions for intake were sometimes given as a range (e.g. “1-2 tablets per day”); where this was the case the minimum amount was selected for daily dose (to ensure at least the minimum dose threshold was met) and the maximum amount was selected for duration (to ensure at least the minimum duration was met). Daily dose was calculated using dose per prescription and instructions for intake and carried forward to calculate duration using the amount of drug prescribed. British National Formulary (BNF) (Joint Formulary Committee, 2017) dose recommendations for major depressive disorder (MDD) differed for adults

(aged 18-64) and the elderly (age>64) therefore age at prescription was taken into account when carrying out dose exclusions. Duration was calculated by summing continuous prescriptions of the same antidepressant. As only the month and not the day of prescription was given, a 31 day margin was given when calculating continuous prescriptions.

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Supplemental Table S1. Antidepressant prescription frequency table for unrelated sample in Generation Scotland: Scottish Family Health Study.

Antidepressant Class	Prescription name	Frequency
Selective Serotonin Reuptake Inhibitors (SSRI)	CITALOPRAM	1218
	ESCITALOPRAM	96
	FLUOXETINE	879
	FLUVOXAMINE MALEATE	2
	PAROXETINE	73
	SERTRALINE	474
	Total	**2742**
Serotonin Noradrenaline Reuptake Inhibitors (SNRI)	DULOXETINE	94
	VENLAFAXINE	195
	Total	**289**
Serotonin Antagonist and Reuptake Inhibitors (SARI)	TRAZODONE HYDROCHLORIDE	62
	Total	**62**
Noradrenaline Reuptake Inhibitors (NRI)	REBOXETINE	4
	Total	**4**
Tricyclic Antidepressants (TCA)	AMITRIPTYLINE	135
	CLOMIPRAMINE HYDROCHLORIDE	26
	DOSULEPIN HYDROCHLORIDE	40
	DOXEPIN	4
	IMIPRAMINE HYDROCHLORIDE	10
	LOFEPRAMINE	31
	NORTRIPTYLINE	8
	TRIMIPRAMINE	2
	Total	**256**
Tetracyclic Antidepressants (TeCA)	MIRTAZAPINE	367
	Total	**367**
Monoamine Oxidase Inhibitors (MAOI)	MOCLOBEMIDE	8
	PHENELZINE	5
	TRANYLCYPROMINE	2
	Total	**15**
Melatonergic Antidepressants	AGOMELATINE	2
	Total	**2**

Supplemental Table S2. Mediation results for association between neuroticism and antidepressant treatment resistance.

	Direct (TR ~ Neuroticism)			Mediation					
	Path c			Path a		Path b		Joint path ab	
Mediator	P_{FDR}	Effect	%	P_{FDR}	Effect	P_{FDR}	Effect	P_{FDR}	%
BRS	**0.041**	**0.006**	**0.6**	**$1.1x10^{-65}$**	**-0.089**	**$6.6x10^{-7}$**	**-0.050**	**$2.0x10^{-7}$**	**0.4**
ToC	**0.00086**	**0.010**	**1.0**	**$1.6x10^{-53}$**	**-0.041**	**0.0053**	**-0.018**	**0.0050**	**0.1**
EoC	**0.0079**	**0.009**	**0.9**	**$1.7x10^{-89}$**	**0.091**	0.064	0.018	0.062	0.2
AoC	**0.00029**	**0.010**	**0.3**	**$1.1x10^{-8}$**	**-0.012**	**$1.2x10^{-9}$**	**-0.095**	**$5.2x10^{-6}$**	**0.1**

Highlighted rows indicate the models with good fit statistics (CFI>0.95, TLI>0.95, RMSEA<0.06). Significant values (P_{FDR}<0.05) are shown in bold. Abbreviations: TR, treatment resistance; ToC, task-orientated coping; EoC, emotion-orientated coping; AoC, avoidance-orientated coping; BRS, brief resilience scale; FDR, false discovery rate; SE, standard error.

Supplemental Table S3. Fit statistics for mediation models with direct association between neuroticism and treatment resistance.

	Fit statistics		
Mediators	CFI	TLI	RMSEA
BRS	0.97	0.96	0.048
Task-orientated coping	1.0	1.0	0.013
Emotion-orientated coping	0.98	0.94	0.036
Avoidance-orientated coping	0.81	0.79	0.071

Highlighted rows indicate models with good fit statistics (CFI>0.95, TLI>0.95, RMSEA>0.06). Abbreviations: BRS, brief resilience scale; CFI, comparative fit index; TLI, Tucker-Lewis index; RMSEA, root mean square error of approximation.

Supplemental Table S4. Factor loadings for the Brief Resilience Scale acting as a mediator for the association between neuroticism and treatment resistance.

Latent Variable	Individual variable items	Beta (SE)	*P* value
BRS=~	BRS1	1.00	
BRS=~	BRS2	0.87 (0.039)	$2.0x10^{-110}$
BRS=~	BRS3	0.95 (0.042)	$3.8x10^{-114}$
BRS=~	BRS4	0.97 (0.042)	$1.2x10^{-118}$
BRS=~	BRS5	0.93 (0.041)	$2.0x10^{-112}$
BRS=~	BRS6	1.04 (0.044)	$8.3x10^{-124}$

Abbreviations: BRS, Brief Resilience Scale; SE, standard error.

Supplemental Table S5. Factor loadings for task-orientated coping acting as a mediator for the association between neuroticism and treatment resistance.

Latent Variable	Individual variable items	Beta (SE)	*P* value
ToC=~	ToC1	1.00	
ToC=~	ToC2	1.25 (0.038)	$4.7x10^{-244}$
ToC=~	ToC3	0.91 (0.030)	$7.7x10^{-210}$
ToC=~	ToC4	1.28 (0.038)	$1.3x10^{-249}$
ToC=~	ToC5	1.14 (0.035)	$1.2x10^{-232}$
ToC=~	ToC6	1.39 (0.040)	$6.0x10^{-267}$
ToC=~	ToC7	1.31 (0.038)	$7.6x10^{-258}$
ToC=~	ToC8	1.17 (0.035)	$1.7x10^{-244}$
ToC=~	ToC9	1.20 (0.036)	$1.4x10^{-243}$
ToC=~	ToC10	1.18 (0.036)	$9.0x10^{-239}$
ToC=~	ToC11	1.16 (0.035)	$1.3x10^{-241}$
ToC=~	ToC12	1.27 (0.037)	$7.1x10^{-259}$
ToC=~	ToC13	1.26 (0.037)	$2.3x10^{-253}$
ToC=~	ToC14	1.28 (0.038)	$1.8x10^{-250}$
ToC=~	ToC15	1.03 (0.032)	$4.0x10^{-221}$
ToC=~	ToC16	1.27 (0.037)	$1.6x10^{-250}$

Abbreviations: ToC, task-orientated coping; SE, standard error.

Supplemental Table S6. Factor loadings for emotion-orientated coping acting as a mediator for the association between neuroticism and treatment resistance.

Latent Variable	Individual variable items	Beta (SE)	*P* value
EoC=~	EoC1	1.00	
EoC=~	EoC2	0.71 (0.026)	$3.7x10^{-161}$
EoC=~	EoC3	1.32 (0.039)	$2.9x10^{-254}$
EoC=~	EoC4	1.40 (0.040)	$2.5x10^{-272}$
EoC=~	EoC5	1.31 (0.037)	$1.3x10^{-267}$
EoC=~	EoC6	0.42 (0.020)	$7.1x10^{-92}$
EoC=~	EoC7	1.31 (0.039)	$6.0x10^{-253}$
EoC=~	EoC8	1.35 (0.039)	$4.0x10^{-269}$
EoC=~	EoC9	1.39 (0.040)	$1.6x10^{-268}$
EoC=~	EoC10	1.07 (0.033)	$5.8x10^{-239}$
EoC=~	EoC11	1.04 (0.032)	$1.3x10^{-231}$
EoC=~	EoC12	1.39 (0.039)	$5.1x10^{-272}$
EoC=~	EoC13	0.92 (0.019)	$2.0x10^{-6}$
EoC=~	EoC14	1.24 (0.036)	$8.0x10^{-254}$
EoC=~	EoC15	1.07 (0.033)	$2.5x10^{-227}$
EoC=~	EoC16	0.91 (0.030)	$1.9x10^{-198}$

Abbreviations: EoC, emotion-orientated coping; SE, standard error.

Supplemental Table S7. Factor loadings for avoidance-orientated coping acting as a mediator for the association between neuroticism and treatment resistance.

Latent Variable	Individual variable items	Beta (SE)	*P* value
AoC=~	AoC1	1.00	
AoC=~	AoC2	1.72 (0.110)	$1.1x10^{-54}$
AoC=~	AoC3	1.22 (0.087)	$7.4x10^{-45}$
AoC=~	AoC4	0.91 (0.077)	$3.8x10^{-32}$
AoC=~	AoC5	1.80 (0.116)	$3.9x10^{-54}$
AoC=~	AoC6	1.81 (0.115)	$1.8x10^{-55}$
AoC=~	AoC7	1.66 (0.108)	$5.8x10^{-53}$
AoC=~	AoC8	0.64 (0.046)	$3.1x10^{-43}$
AoC=~	AoC9	2.45 (0.148)	$1.8x10^{-61}$
AoC=~	AoC10	2.52 (0.153)	$6.1x10^{-61}$
AoC=~	AoC11	1.23 (0.090)	$6.8x10^{-43}$
AoC=~	AoC12	1.91 (0.119)	$5.7x10^{-58}$
AoC=~	AoC13	2.34 (0.143)	$4.3x10^{-60}$
AoC=~	AoC14	1.27 (0.085)	$3.8x10^{-50}$
AoC=~	AoC15	0.98 (0.077)	$2.2x10^{-37}$
AoC=~	AoC16	1.01 (0.081)	$8.4x10^{-36}$

Abbreviations: AoC, avoidance-orientated coping; SE, standard error.

Appendix 4. Publications.

FIRST AUTHORSHIPS

Wigmore EM, Clarke TK, Howard DM, Adams MJ, Hall LS, Zeng Y et al., (2017): **Do Regional Brain Volumes and Major Depressive Disorder Share Genetic Architecture? A study in Generation Scotland (n=19,762), UK Biobank (n=24,048) and the English Longitudinal Study of Ageing (n=5,766).** *Transl Psychiatry*. **7**, e1205; doi:10.1038/tp.2017.148.

Wigmore EM, Hafferty JD, Hall LS, Howard DM, Clarke TK, Fabbri C et al,. (2017): **Genome-wide association study of antidepressant treatment resistance in a population-based cohort using health service antidepressant prescription data with meta-analysis with GENDEP.** In submission.

Wigmore EM, Navrady LB, Hafferty JD, Clarke TK, Campbell A, Thomson PA et al., (2017): **Moderation and mediation effects of stressful life events, resilience and coping style in antidepressant resistance.** In preparation.

Wigmore EM, Clarke TK, Porteous DJ, Nicodemus KK, McIntosh AM (2017): **Toward genetic stratification of neuropsychiatric disorders.** In preparation.

CO-AUTHORSHIPS

McIntosh AM, Hall LS, Zeng Y, Adams MJ, Gibson J, Wigmore E et al., (2016): **Genetic and Environmental Risk for Chronic Pain and the Contribution of Risk Variants for Major Depressive Disorder: A Family-Based Mixed-Model Analysis**. *PLoS Med*. 13(8): e1002090.

Whalley HC, Adams MJ, Hall LS, Clarke TK, Fernandez-Pujals AM, Gibson J, Wigmore E et al., (2016): **Dissection of major depressive disorder using polygenic risk scores for schizophrenia in two independent cohorts**. *Transl Psychiatry.* 6(11): e938.

Reus LM, Shen X, Gibson J, Wigmore E, Ligthart L, Adams MF et al., (2017) **Association of polygenic risk for major psychiatric illness with subcortical volumes and white matter integrity in UK Biobank.** *Sci Rep.* 7: 42140.

Gibson J, Adams MJ, Clarke TK, Hall LS, Fernandez-Pujals AM, Wigmore EM et al., (2017): **Assessing the presence of shared genetic architecture between Alzheimer's disease and major depressive disorder using genome-wide association data**. *Transl Psychiatry.* 7(4): e1094

Howard DM, Adams MJ, Clarke TK, Wigmore EM, Zeng Y, Hagenaars SP et al., (2017): **A haplotype-based association analysis of general cognitive ability in Generation Scotland, English Longitudinal Study of Ageing and UK Biobank.** In submission.

Howard DM, Clarke TK, Adams MJ, Hafferty JD, Wigmore EM, Zeng Y et al., (2017): **The genetic stratification of major depressive disorder into distinct subgroups using Buhmbox.** In preparation.

Hall LS, Adams MJ, Arnau-Soler A, Clarke TK, Howard DM, Zeng Y … Wigmore EM et al., (2017): **Genome-Wide Meta-Analyses of Stratified Depression in Generation Scotland and UK Biobank.** *BioRxiv*: doi: https://doi.org/10.1101/130229

Howard DM, Adams MJ, Shirali M, Clarke TK, Marioni RE, Davies G **…** Wigmore EM et a., (2017): **Genome-wide association study of depression phenotypes in UK Biobank (n = 322,580) identifies the enrichment of variants in excitatory synaptic pathways.** *BioRxiv.* doi: https://doi.org/10.1101/168732

Navrady LB, Wigmore EM, Clarke TK, Zeng Y, Adams MJ, Chan SWY et al., (2017). **Genetic and environmental contributions to psychological resilience and coping**. In preparation.